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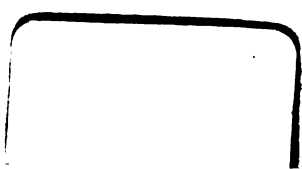
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1903.—No. 11.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

BIOLOGICAL LABORATORY.

ENTOMOLOGICAL DIVISION.

BULLETIN No. 1.

PRELIMINARY BULLETIN ON INSECTS OF THE CA

PREPARED ESPECIALLY FOR THE BENEFIT OF FARMERS.

By CHARLES S. BANKS,

Entomologist, Bureau Government Laboratories.

MANILA:

BUREAU OF PUBLIC PRINTING.

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LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., September 14, 1903.

SIR: I have the honor to transmit herewith "A Preliminary Bulletin on Insects of the Cacao," by Mr. Charles S. Banks, Entomologist, Bureau of Government Laboratories.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

HON. JAMES F. SMITH,
Acting Secretary of the Interior, Manila, P. I.

11

ILLUSTRATIONS.

Cacao mealy bug	Frontispiece.
	Facing page.
FIG. 1. <i>a, b, c</i> , Black ant, larva, and pupa	9
2. Wood attacked by white ants	10
3. Cicada, adult	10
4. Cicada, wing venation	10
5. Cicada, egg-laying apparatus (ovipositor)	11
6. Cicada, nearly full-grown nymph	11
7. Cicada, forefoot of nymph	11
8. Cicada, appearance of twigs after oviposition (from U. S. Dept. of Agr. Div. Ent., Bul. 14, n. s.)	14
9. Cicada, old wounds in tree after one year	14
10. <i>Anomala</i> , species related to that on cacao	17
11. <i>Anomala</i> , larvæ of species found on cacao	17
12. <i>Anomala</i> , larva in pupal cell	17
13. <i>Cerambycid</i> , adult	19
14. <i>Cerambycid</i> , larva	19
15-17. Branches killed by <i>Cerambycid</i> larvæ	20
18. 18 <i>a, b</i> , <i>Cerambycid</i> , burrows of larvæ in wood	21
19. Bag used to protect the upper parts of the tree	23
20. Bags for the protection of the lower part of the tree	23
21. Termite, adult female distended with eggs	25
22. Termite before distention with eggs, much enlarged	25
23. Termite, cells in which the young are reared	25
24. Termite, cross section of the queen cell	25
25. <i>Psocidæ</i> clustered on the trunk of the cacao tree and indi- vidual <i>Psocids</i>	26
26. Twig girdler, work on twig 26 mm. in diameter, enlarged	27
26 <i>a</i> . Twig girdler, work on twig 13 mm. in diameter, reduced	27
27. Orthopteron, work on branches, showing scar	27
28. Orthopteron, work on branches, showing eggshells in place	27
29. Orthopteron, small scar with eggs	28
30. Plant lice, stages from larva to adult	29
31. Plant lice, fruit distorted by lice	30
32. Thrips, leaves curled by the insect in its attacks	30
33. Thrips, adults enlarged	30
34. Large yellow scale, adult enlarged and eggs at <i>b</i>	31
35. <i>Sphindidiæ</i> , adult, showing markings of wing covers, enlarged	31
36. <i>Euclidæ</i> , full-grown larva	33
37. <i>Euclidæ</i> , pupa	33
38. Slug caterpillar, damage to leaves	34

	Facing page
FIG. 39. Slug caterpillar, larva	35
40. Slug caterpillar, cocoon from which the adult has escaped ...	35
41. Slug caterpillar, another form, larva and parasite	35
42. Slug caterpillar, another form, work on leaves	35
43. Chrysomelidæ, full-grown beetles	36
44. Chrysomelidæ, larva of related species (redrawn from Com- stock)	42
45. Sisí scale insect, showing the insect on the leaf and enlarged ..	42
46. Alingayo, adult and larva and pupa	42
47. Nest of alingayo	42
48. Amomó-ong, adult	43
49. Nest of amomó-ong	43
50. Red corsair	44
51. Ant-lion, two views of nest	46
51a. Ant-lion, two views of larva	46
52. Praying mantis, egg mass	47
53. Praying mantis, young	47
54. Praying mantis, adult	47
55. Orb weaver spider, crescent variety	48
56. Jumping spider and ant-like form	48
57. Cacao pods attacked by rats	49
58. Cacao leaf spot	52
59. Cacao pod fungus, spotted form	52
60. Cacao pod fungus, blight form	52

A PRELIMINARY BULLETIN ON INSECTS OF THE CACAO, PREPARED ESPECIALLY FOR THE BENEFIT OF FARMERS.

By CHARLES S. BANKS,
Entomologist, Bureau of Government Laboratories.

This bulletin is prepared particularly with a view to its practical value to the farmers of the Philippines in the cultivation and protection of an industry which promises much in the future for these Islands.

As this bulletin is the result of only three months actual investigation of cacao insects, it does not in any way cover all the insects which are directly or indirectly associated with the growing of cacao.

Much has yet to be learned of the habits and life-histories of many of the insects treated herein, owing to the fact that the periods of their transformation extend over a year or more. The publication of the material at this time is in response to an urgent demand upon the part of growers for information that will help them in combating the more serious pests.

All the illustrations, where not otherwise credited, are from photographs made by myself or by the Government photographer under my direction, or are from original drawings made by myself or under my supervision by Juan de Guzman and José Garcia.

The frontispiece was drawn by me from nature, partly in the field and partly from material brought back from my trip.

I wish to acknowledge my personal thanks to Sr. Don Juan A. Araneta, of the hacienda "Louisiana," Maao, Occidental Negros, for the many ways in which he aided me in the work of investigation which I carried on upon his plantations.

C. S. B.

MANILA, P. I., *August 28, 1903.*

CACAO INSECTS.

Up to the present time, so far as search has revealed, very little has been published upon the subject of economic entomology in the Philippine Islands. In the several admirable works on the entomology of the Archipelago, we find not only that the economic side has been entirely neglected, but also that the same thing is true from the biological standpoint. The only attempts made in the past have been to determine the names of specimens, in most cases collected by one man and classified by others. For even the most common species of insects, few or no data are given regarding their habits, life-history, or relative abundance at certain periods of the year or in stated localities.

In preparing this brief preliminary bulletin on the insects of the cacao, I realize that I am but hinting at some of the more important pests which have been encountered during a comparatively short period of the year; so that all the conditions as they would occur consecutively during the course of twelve months, and undoubtedly changing slightly with each succeeding year, are not now noted, nor are all the subjects mentioned treated exhaustively, the object being at the present time only to set forth as clearly as possible some of the most common and more destructive insects, with practical suggestions for the prevention or treatment of their ravages. At the same time it is proposed to mention a few of those insects, which, because of their predatory habits in feeding upon the injurious forms, should be considered as beneficial to the farmer.

Perhaps one of the most valuable crops produced in the Philippine Islands in proportion to the quantity raised, is the cacao bean, the product of a tree of the family *Sterculiaceæ* and botanically known as *Theobroma cacao*. The tree averages from 4 to 12 meters in height, and, as grown in these Islands, usually assumes a somewhat oval form in its mode of branching, like the pear tree of the United States.

Like nearly all other plants, when brought into cultivation, it is subject to many diseases and the attacks of a large number of

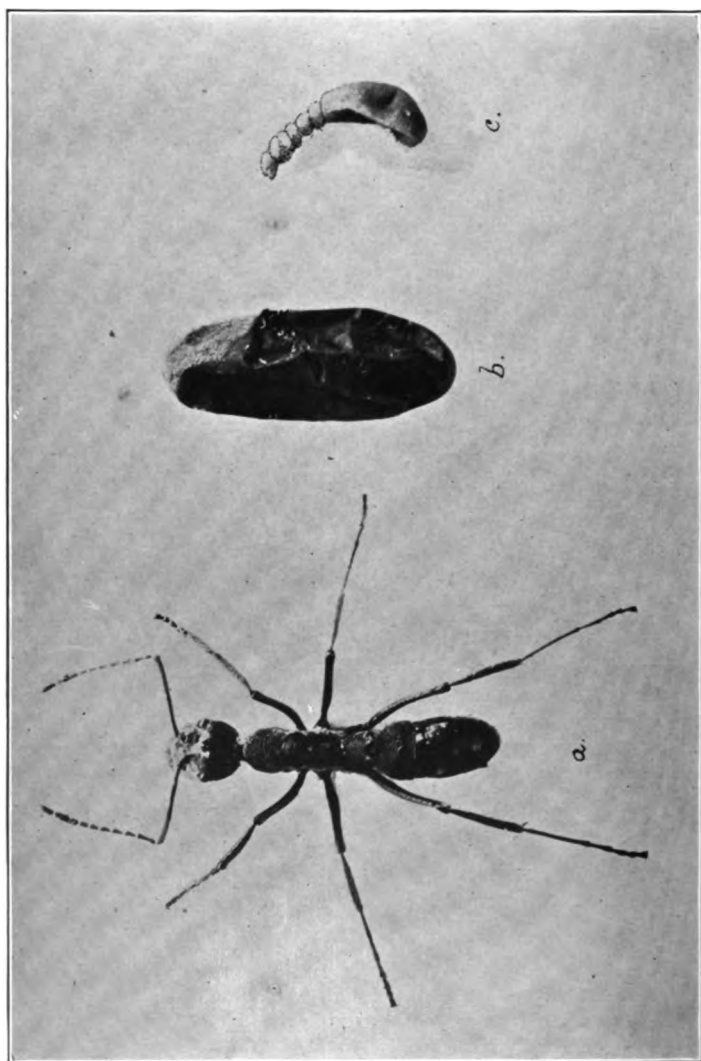


FIG. 1.

insects. This is more or less true in all parts of the world where it is grown, in Venezuela, Trinidad, Java, and particularly, it would seem, in the Philippines.

In this bulletin some of the injurious forms of insects will be mentioned, together with what has been learned of their life histories up to the present time. As it is very necessary to know the most susceptible stage of an insect's life in order to know what is the easiest remedy to apply to it, we can readily see the necessity for becoming familiar with its life-history. It is hoped that further observations will enable me to treat the subject more exhaustively at a future time.

In suggesting remedies, those are given which have been found most effectual for similar pests in the United States, but the effort has been made to so modify them as to make them fit local conditions.

For convenience, the subject has been divided according to the part of the plant attacked. Thus, beginning with the roots, we will in order discuss the insects of every part of the tree, giving their habits and mentioning the best means to be used against them.

INSECTS ATTACKING THE ROOTS.

BLACK ANTS.

The principal insects at present known to attack the roots are a species of large, ferocious ant, a species of *Cicada*, and the grub of a beetle belonging to the genus *Anomala*, but not yet identified.

The ants are black and are characterized by the ability to inflict a very severe sting. The abdomen is constricted between the first and second segments, the first segment having two backward-pointing spines on the upper part. The head, thorax, and first and second abdominal segments are beautifully corrugated. The legs and antennæ or feelers are very long. The light-brown cocoons, made of a silk paper, are usually very abundant in the nests, which are located at the base of the tree among the larger roots. Within these cocoons may be found the white grubs, which are shaped very much like a long-necked gourd, the head being at the smaller end. (See fig. 1 c.) The larvæ or grubs, before spinning their cocoon, and the eggs are simply deposited by the workers in any convenient part of the nest. The adults are the only injurious forms of these insects. They gnaw the bark from the large roots,

thus inviting decay, and making an opening for the entrance of the insidious white ant, another very serious enemy of the cacao, belonging to the genus *Termes*. The latter is called *anay* in Visayan and Tagalog, and is probably the most serious insect pest in the Philippines, destroying, as it does, nearly every conceivable class of material except articles made of metal. It has hitherto been supposed to attack only woods which had been previously cut, but in the work of investigation on cacao insects it has been conclusively proven that it also attacks the living tree, at least in the case of cacao. (See fig. 2.)

In the United States the members of the genus *Cicada* are restricted to not more than five species. In the Philippines there are several, some being large and black and others grey, while still another species is of a very light yellowish green. A singular thing about the individuals of the genus is that the males give forth a strident noise, produced by means of two drum-like organs on the lower side of the first segment of the abdomen. Unlike the members of this genus in America and Europe, which generally "sing" in the hottest days of summer, the individuals found in the Philippines almost invariably reserve their serenade till the falling of darkness, when their strident notes may be heard on every hand, especially near wooded lands.

All observations thus far made upon this interesting insect would lead me to infer that the habits and life history are the same in the Philippine Islands as in other parts of the world, particularly so in the case of the dog-day cicada in the United States.

Here the species may be found at all times, and the insects are so common as to be used as playthings by the native children, who capture them to make them sing. In Visayan they are called "*Ceriritan*" and in Tagalog "*Culiclic*."

The adult of the species which attacks the cacao tree measures 42 mm. to the tip of the wings, which project 13 mm. beyond the tip of the abdomen. The body is robust, somewhat conical, and is composed of a series of very regular segments, which may be more easily distinguished on the abdomen than on the thorax. (See fig. 3.) The insect has four very beautifully marked, transparent wings, the fore ones being much larger than the hind. The venation is shown in fig. 4. When at rest the insect's wings are laid over the abdomen, roof-like. The *Cicada* has a swift but erratic flight. This is due to the lack of coördination between the



FIG. 2.

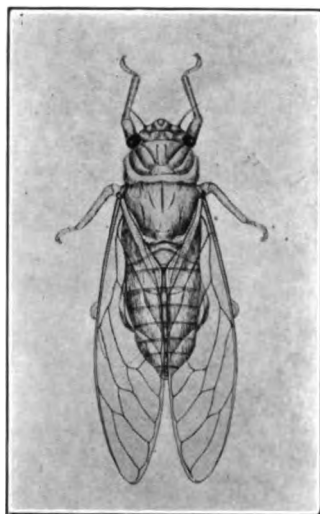


FIG. 3.

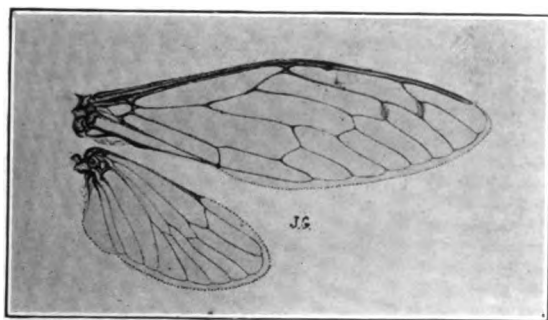


FIG. 4.

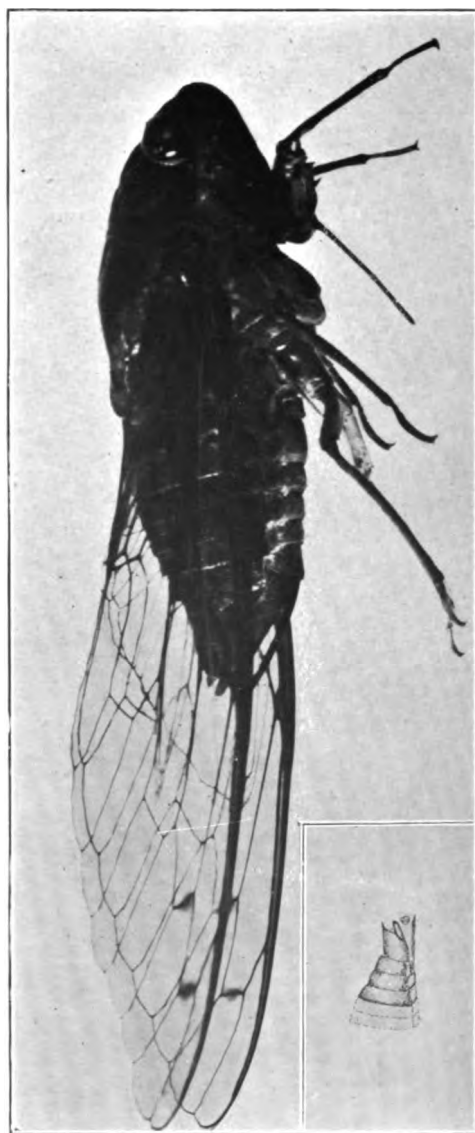


FIG. 5.

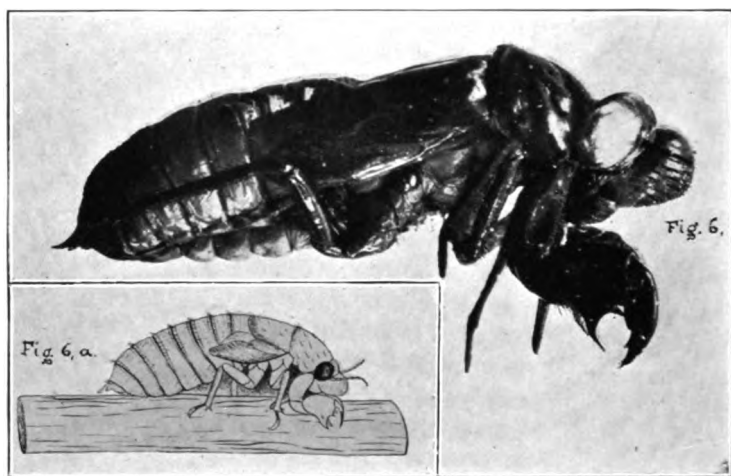


FIG. 6, FIG. 6 a.



FIG. 7.

wing muscles of the opposite sides of the body. By holding the insect between the thumb and the forefinger, so that the wings are free to move, it will be seen that the opposite pairs move alternately.

The insects of this genus pass through a very remarkable series of changes from the egg to the adult stage. The total duration of the metamorphosis has not yet been determined, but it is safe to estimate that in this region the time occupied is not less than eighteen months. In America one species nearly related to this lives from thirteen to seventeen years in the ground before reaching maturity, while another completes its transformation in two years.

The female *Cicada* is provided with a very peculiar ovipositor or egg-laying apparatus (fig. 5), by means of which she slits the twigs of young branches of the cacao. Inasmuch as the wood of the cacao is comparatively soft, she finds no great difficulty in placing her eggs in branches which are larger than those ordinarily found with *Cicada* eggs in the United States. Within from four to six weeks from the time the eggs are laid, the young hatch. They are tiny white creatures which resemble very much the full-grown nymph, except in size. Their forefeet are adapted for digging in the ground, and they, dropping from the twigs, begin immediately to burrow down to the tender rootlets of the plant, where they settle and insert their beaks for sucking the juices of the roots. Fig. 6 shows the nearly full-grown nymph, and fig. 7 shows its forefoot. The larva or nymph forms a dirt cell around the spot where it decides to remain. This it does with its forefeet, using them as a mole would, and packing the earth at the rear and above it with its other legs. Thus is made a subterranean cavity where this little enemy of the roots of the cacao may live and feed.

The mouth of the insect is of the sucking class—that is, the various parts are modified to form a lancet for piercing the epidermis of the plant upon which it feeds, and a tube-like structure through which the sap is drawn up into the mouth cavity, whence it is conveyed to the stomach.

When very young, the larvæ may be found not more than 10 or 12 cm. below the surface of the ground, but as they grow larger they gradually work their way downward. In some cacao trees they have been found as deep as 80 cm. below the surface, clinging tenaciously to the roots by their beaks and their legs.

They are not at all able to defend themselves, nor to escape from any kind of enemy which might come upon them.

These insects do not change their form to a very marked degree in passing from the larval to the pupal and the adult stages, as do butterflies, beetles, and flies. Just previous, however, to their assuming the adult form, there appear tiny wing pads on the sides and within these are the wings in embryo. These pads appear in the second moult previous to the adult stage. (See fig. 6, which shows the wing pads lapping back upon the abdomen.)

When the insect is ready to assume its final or winged form it comes up out of the ground, leaving a tunnel behind, which may often be found when digging out trees. These tunnels are sometimes partitioned off and used as nests by a certain species of small tarantula, which will be mentioned below.

Upon reaching the surface of the ground the pupa of the *Cicada* climbs to some convenient place, usually the trunk of the cacao tree or the small scions which spring up around the parent tree, and there it clings, awaiting the final change, which consists in the bursting of the pupal skin and the emerging of the adult insect. This takes place as follows: The skin of the pupa splits longitudinally on the back, from between the eyes to the posterior edge of the thorax. The insect within this shell begins a forward wriggling motion and soon the head is free from the casing. The forelegs, which in the larval and pupal stages are much-shortened hooked claws, come forth from the pupal case, long, slender, and fitted for walking instead of digging. The insect grasps some projecting point upon the bark where it is resting, with these yet feeble claws, and thus is aided in its exit. The second pair of legs soon come forth, then the wings, which are very much doubled up and are very soft, are drawn out from the shell. At this stage the animal rests a while, apparently nearly exhausted. When it has thus rested, the insect makes its final attempt and the hind legs and abdomen are withdrawn. It now simply remains upon the tree, and the doubled and delicate wings begin to expand until they have reached their full length. It is supposed that this expansion is due to air pressure within the body. They are now very thin, of a beautiful translucence and very soft, so that the least breeze causes their fluttering. The body of the insect is also very soft and may be easily crushed. It is of a very pale, pinkish color, but soon turns grey, evidently owing to the action of the sunlight, all the

characteristic markings coming out upon its surface. Within an hour, seldom less, the insect begins to move its wings as if testing them, and now the slightest movement on the part of the observer will cause it to quickly take wing, giving a short clicking sound as it flies away. If the insect be a male, it at once makes its characteristic "creet," a noise familiar to all, as it starts to fly. If it be a female, it remains in silence. It is not definitely known how long a period elapses from the emergence of the insect from the ground, until it mates and begins the work of laying its eggs. Further observations will be necessary to determine this.

The principal injury to the cacao from these insects consists in the lacerating of the young wood by the females in placing their eggs, and in the damage done to the tender rootlets by the larvæ and pupæ. The former not only debilitates the tree from the mechanical injury, but also opens a path by which other injurious insects may enter the tree to do their damage. This is no inconsiderable feature of their harm, especially when they attack the larger twigs and stems, because it is more frequently upon the larger stems that *Scolytids*, *Cerambycids*, and other beetles do their work.

When fifty or more of the larvæ attack the young roots and rootlets, the principal food-getting organs of the tree, they not only use up the moisture which the roots have already taken up, but they likewise so mutilate and debilitate the latter as to impair the functions of moisture gathering. Of the several cacao trees which the writer dug up, none were more than 5 m. high and in no case did their roots extend more than 95 cm. below the ground surface, and as the pupæ of *Cicada* were found as low as 80 cm. it can be readily seen that they command practically the whole of the root area.

In the matter of combating these insects, several methods suggest themselves. As it is practically impossible in a large plantation to use preventive measures entirely, those means which also look to the extermination of the already established pests must be used.

The pupæ, when emerging from their ground retreats, are entirely helpless, and they, as well as the newly transformed adults, are easily captured upon the daily visits to all the trees by the watchers and workers in charge; in fact this could very easily be done by children, who seem particularly expert in capturing the in-

sects which they like to make "sing." Even the full-fledged adults of several days may be captured upon the trees at dusk, when the males begin their concerts, and the females are close by to listen. During midday the insects may sometimes be caught upon the tree trunks, but are more wary and harder to take.

Certain species of birds are known to be enemies of the *Cicada*, at least in the Island of Negros. Repeatedly the writer, when in the cacao plantations, has heard the uneasy creet of the *Cicada*, and following with his eye the direction of the sound, has seen a bird carrying off the insect. The species could not be ascertained, but from its general size and form, it undoubtedly belonged to the Shrikes or *Laniidae*. This should lead the grower to carefully protect all birds which have the inclination to visit or live in the plantation of cacao, as being beneficial, not only in the destruction of the *Cicada*, but also in ridding the trees of other injurious insects like scale-insects, mealy-bugs, and caterpillars of various species.

If the insects are captured upon emerging they will have no opportunity to lay their eggs, but as this can not be done in all cases, a close daily survey of the trees at the time when the adults of *Cicada* are beginning to be numerous will reveal the work which they do on the twigs. The characteristic appearance of the twigs after eggs have been deposited in them is shown in fig. 8. It was claimed a number of years ago that the *Cicada* only deposits her eggs in dead twigs, but observation has since proven that this is not the case, she invariably choosing live wood for this purpose. The short time during which the eggs remain in the slit would not be sufficient for the growth to crush them, and moreover, the growth of a twig which has been lacerated by the ovipositor of the *Cicada* is away from the wound, causing the latter to gape within a year from its formation, as shown in fig. 9. If these twigs are carefully removed by means of a very sharp knife, and if they are afterwards destroyed by burning, while the eggs are yet unhatched, much can be done to lessen the number of *Cicada* attacking the trees.

If a twig, into which eggs have been newly laid, be examined by cutting it open longitudinally, the white eggs will be found arranged very regularly in the cavities which have been made to receive them. If the eggs have recently hatched, their transparent and shrivelled shells will be all that can be found. After a few days

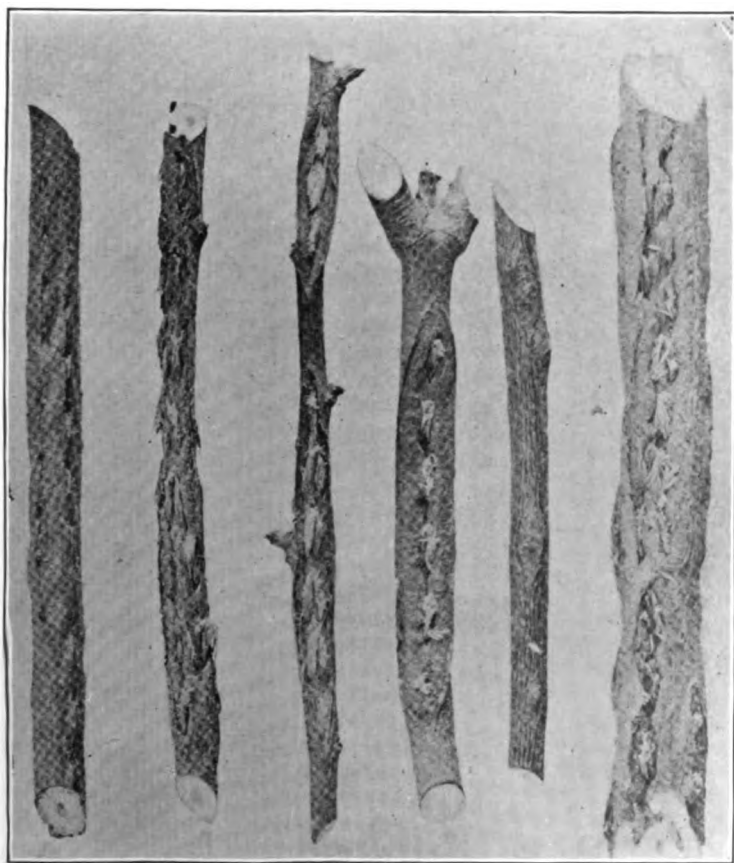


FIG. 8.—From Bul. 14, N. S., U. S. Dept. Agr., 1898.



FIG. 9.

these shells will have disappeared, being eaten by the numerous ants which constantly swarm on all parts of the tree. It may be that occasionally the eggs are found by the little red ants and eaten before they are hatched, but as the fibers of wood left by the boring of the female *Cicada* pretty effectually close the cavities, this is not probable.

During the months from January to April, 1903, all sizes of larvæ and the pupæ were found when cacao trees were dug out. During this same period the adults were fairly abundant, as were the cast skins of the pupæ on the trees. It is therefore probable that the period of greater abundance of the adults is during the latter part of the year, probably in October and November.

The question of the killing of larvæ after they have once entered the ground is a most difficult and serious one. There are few substances which have sufficient penetrating power to enter the soil and be effectual against the larvæ without endangering the young roots of the trees. Probably one of the most useful of these is carbon bisulphide, an extremely volatile transparent liquid resembling highly refined petroleum, but having, when not pure, an extremely disagreeable odor. It is highly inflammable and heavier than the air, and therefore sinks readily into holes or crevices in the ground. Its extreme inflammability renders great precaution necessary when handling it. It must not be kept in houses where lights are used, nor must it be left in large quantities in bright sunlight, and the vessel used to contain it must be capable of being sealed. The fumes of this chemical, while not actively poisonous when breathed by human beings in limited quantities, are extremely disagreeable and are productive of headache. If inhaled for a moderate length of time they cause suffocation. The substance should be handled only by those who are thoroughly acquainted beforehand with its properties, and then only by those who can be trusted to carry out instructions concerning its uses. It should be kept where it can not be reached by children, and should be labeled "Poison." Glass-stoppered bottles or tin cans having the best quality of cork stoppers are the best receptacles for carbon bisulphide.

It is possible that gasoline or naphtha would in a measure serve the same purpose as carbon bisulphide, but no experiments have yet been made to verify this supposition. Either of the liquids would have the advantage of cheapness over carbon bisulphide.

At present the price of this chemical in the Philippines is such

that the general use of it as an insecticide is almost out of the question. In the United States, when bought in large quantities, it may be had for about 15 cents per pound. By the single pound it costs about 25 cents, gold. In the drug stores of Manila it may be bought at 75 cents, gold, per pound; or 55 cents, gold, in quantities of from 50 to 100 pounds. It is hoped that with this, as with other important insecticides, some plan may be devised to enable the farmer here to procure them at a lower cost.

The most effective method for the application of bisulphide to trees for the killing of insects attacking the roots, and the one which is least dangerous to the tree, is as follows: The trees may be treated successively by one or several men. An instrument should be used by which a hole 50 or 60 cm. deep and 2 cm. in diameter may be bored into the earth, about half a meter from the crown of the tree. There should be three of these holes equidistant around each tree, and into each should be poured not more than 25 c. c. of the carbon bisulphide. The hole, after introducing the liquid, should be immediately stopped up with wet earth or thick wet cloths, and left in this condition for several hours. The cloths can then be taken away, or the ground raked or leveled if earth only has been used. These measures can be tried at any time, but preferably just after a period when a large number of twigs of the trees have been found lacerated by the female *Cicada*, as in all probability the eggs will have been but recently hatched and the larvæ therefore but a short distance below the ground.

When *Anay* or ants are found working below the crown of the tree, similar measures for their extermination may be employed. A dampened canvas or other air-tight cloth may be placed around the base of the tree, in the form of a conical tent with a broad base. After earth has been packed around the base, a small quantity, from 15 to 20 c. c., of carbon bisulphide may be poured upon the ground within the tent and the whole thus left for half an hour. If rightly applied this remedy will kill all insects within the inclosed area without doing damage to the tree itself. The method here described applies only to trees which have attained their full growth, and just after the bearing season. With smaller ones a proportionately smaller amount of the bisulphide should be used.



FIG. 10.

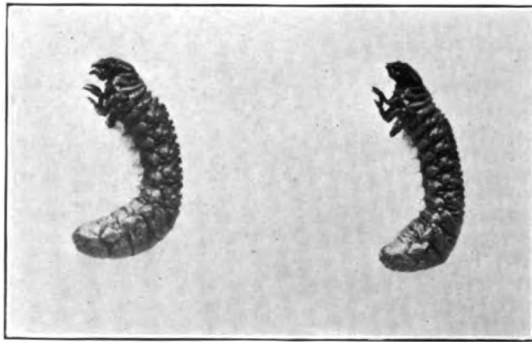


FIG. 11.



FIG. 12.

WHITE GRUBS.

Not only will the method described for the killing of the larvæ and pupæ of *Cicada* prove effectual for these insects, but it will also be equally so against the white grubs so commonly found among the roots of the cacao tree.

These white grubs are the larvæ of one of the *Lamellicorn* beetles and belong to the genus *Anomala*, the adults of which have been found in cacao plantations in some abundance. In general, the habits of all beetles of this genus are the same. The adult of a very common species is shown by fig. 10, and the larvæ of the form found in the cacao root by fig. 11. It is not known how or where the eggs are laid, but they are probably deposited in the débris which naturally collects around trees which are ill cared for. The larvæ, upon hatching, have but a very short distance to go, in the case of the cacao, before they find the young tender rootlets upon which they feed with avidity.

These larvæ can be very easily recognized. The form of the body is characteristic. When found they are invariably curled up, assuming this position as a means of defense. The full-grown larva measures 3 cm. in length and about 5 mm. in diameter. The long, rather slender, yellowish, hairy legs are bent forward at the first joint. In crawling the insect uses them very awkwardly. The head is ochre-yellow, shiny, and covered rather sparsely with stout bristle-like hairs. Examination with a strong hand-lens reveals the fact that there are no eyes, at least none externally. The dark-brown jaws or mandibles are well fitted, by their form and position, for cutting off bits of the roots upon which the animal feeds.

The body has a somewhat corrugated surface, and upon the back of each segment there is a transverse area covered with fine, brown, very short bristles, which aid the insect in burrowing into the ground. The rear four segments of the abdomen are somewhat broader than the others and are of a darker color, owing to the mass of excrement contained in the alimentary canal.

That these insects do much damage to cacao is evident from their abundance and the fact that they are related to species which are very injurious wherever found in other parts of the world. Living as they do for the greater part of their lives below the

surface of the ground, where cultivation of the plant will not disturb them, they form one of the hardest classes of insects to combat. The effects of their work are not apparent until the weak and dying condition of the tree, and its inability to bear fruit, tell the grower that it is being killed by some unseen insect enemy. The full-grown insect, a beetle, is shown in fig. 10. It measures from 9 to 12 mm. in length, is of a shiny greenish-brown color, and the wing-covers are very much rounded. Fine striations, running longitudinally upon the wing covers, heighten its sheen. The beetles are usually found upon the leaves or in crevices in the bark, where they appear to be always in hiding. These beetles are very peculiar in their habits, invariably simulating death when they are disturbed; they then drop to the ground, where they lie perfectly motionless among the rubbish around the tree until the disturbance has passed. If care be used, a very slight jarring of the tree will cause them to drop from it to the ground, where, if there be no débris, they may be seen and killed. A word of caution, however, should be given as to jarring the trees. Perhaps no tree cultivated is so susceptible to ill treatment as the cacao. The fruit, because it is so large and heavy, may be shaken off by even a strong breeze, and it is therefore evident that jarring, when the tree is in fruit, will prove most disastrous to it. Only such trees as are just out of fruit or in the blossom season should be treated in this manner, and the jarring should be done with the palm of the hand; a gentle blow being delivered, and only repeated two or three times for each tree. Anything more severe than this will result in disaster.

For killing the larvæ of the beetle above described the same methods as those employed for killing *Cicada* larvæ may be used.

When this insect is ready to change to the pupal stage it builds a cell, composed of compacted earth. This cell is somewhat larger than the larva, and, as the latter shrinks considerably just previous to the pupal stage, there is sufficient room for it to effect the final change to the adult, allowing for the proper expansion of the wings and wing-covers. While in this pupal cell the insect appears as in fig. 12.

While birds, particularly crows, feed most readily upon all forms of white grubs when they are exposed in fields by plowing, it is very doubtful if they perform any appreciable service in the destruction of the *Anomala* unless it be that they pick the insects from the trees when they have reached the adult stage. Therefore,

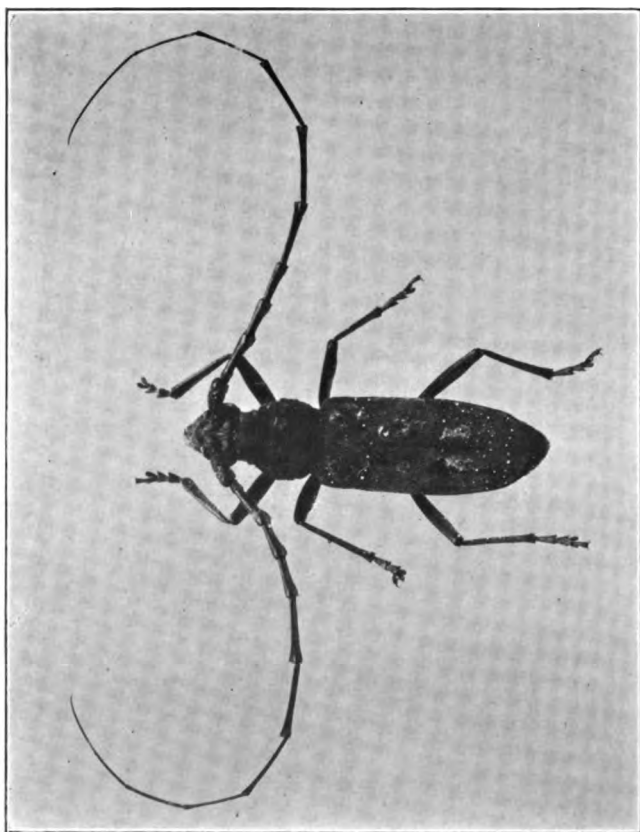


FIG. 13.

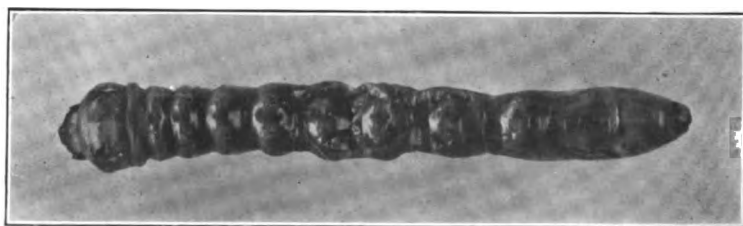


FIG. 14.

all efforts against these pests will consist of the destruction of the larvæ at the time of application of remedies for the *Cicada* and the jarring of the beetles from the trees as described above.

INSECTS ATTACKING THE TRUNK.

FLAT-HEADED BORER.

Of all the cultivated plants observed in the Philippine Islands, perhaps none has been found which suffers more from insect attacks on the trunk than does the cacao. The insect which is of first importance is the flat-headed borer of the cacao. It belongs to the family of long-horned borers, the *Cerambycidae*, an adult of which is shown in fig. 13. The damage by this insect is much greater than that produced by any other insect attacking the cacao, inasmuch as it not only works for a long period unseen in the trunk, but finally effects the death of the tree, and the damage is hardly apparent to a casual observer until its work is completed.

Ninety per cent of all trees examined in the Island of Negros were found more or less completely damaged by this insect. The mode of attack and the results are so characteristic that when once described they will be noticed by the most careless observer, and will be always remembered as the work of this particular insect.

Going through a cacao plantation at certain periods in the year, especially in April and May, one will frequently notice at the bases or upon the trunks of the trees a kind of coarse fibrous sawdust. This may be of a light wood color, or of a very dark mahogany red if there has been a recent rain. One who is not acquainted with the facts might well suppose that this has been produced by the gnawing of some small animal like a rat, though upon closer examination it will be seen that the fibers are too regular to have been thus produced. If the bark be examined carefully one will soon find a small hole of irregular form, about 1 cm. in diameter, from which there appears to be exuding more sawdust, usually of a dark color and wet if recently pushed out of the burrow by the insect. If a hooked instrument or a knife be used to carefully remove the flakes of dead bark found around the hole, there will be found more of the same material underneath. If the search be continued, following the line of a now well marked burrow, the chances are that at its end, whether just below the bark in the sapwood or within a deeper burrow toward the heart of the tree will be found a large, yellowish grub with darker head and still darker

brown jaws. The body is larger toward the head and somewhat flattened, giving the insect the name flat-headed borer. The body segments are well defined, as shown in fig. 14. The darker color of the hinder part of the body is due to the fact that the digested wood becomes colored by the juices in the alimentary canal of the larva. It would be well to state here that the fibrous masses of wood found in connection with this insect have not been used as food, but were simply cut away in the process of making the pupal cell and in filling up its entrance, in order that the grub might not be disturbed in its transformations to the pupal and adult stages.

This larva may well be described as a footless grub; one which is perfectly helpless when removed from its burrow, for when once taken out it is not only at the mercy of predacious insects like ants, but is of itself so helpless that it would soon die from lack of moisture before it could reach a place of safety.

The full-grown larva measures 40 mm. in length and about 4 mm. in diameter at the middle of the body. Its powerful jaws or mandibles enable it to feed readily upon the hardest parts of the wood in which it lives, and specimens have been found which have excavated tunnels 50 cm. long, in addition to the irregular blotch-like cavities which are formed just beneath the bark at the beginning point of the attack.

The habits of this insect are very similar to those of related species in other countries, as the maple borer, *Plagionotus speciosus*; the sawyer, *Monohammus confusor*; the round-headed apple borer, *Saperda candida*; the oak pruner, *Elaphidion villosum*. The female lays her egg in a small puncture in the living bark of the tree, and as soon as the young grub hatches it begins its work of tunneling the bark, the tender growing wood, and eventually the harder portions of the tree. Were it true that a single borer did its work in the trunk, the damage, though considerable, would not be so serious a menace to the life of the tree. But when several borers appear simultaneously in different parts of the trunk they soon succeed in girdling it, after which the tree is sure to die. Figs. 15, 16, and 17 show several large-sized branches killed in this manner. The grubs having injured one side of the tree, the latter puts forth its strength in efforts to heal the wound, causing an abnormal growth to bulge over the bare space left by the insect attack. The wood and bark increase in thickness on the opposite side. Other grubs attack this part, which is particularly

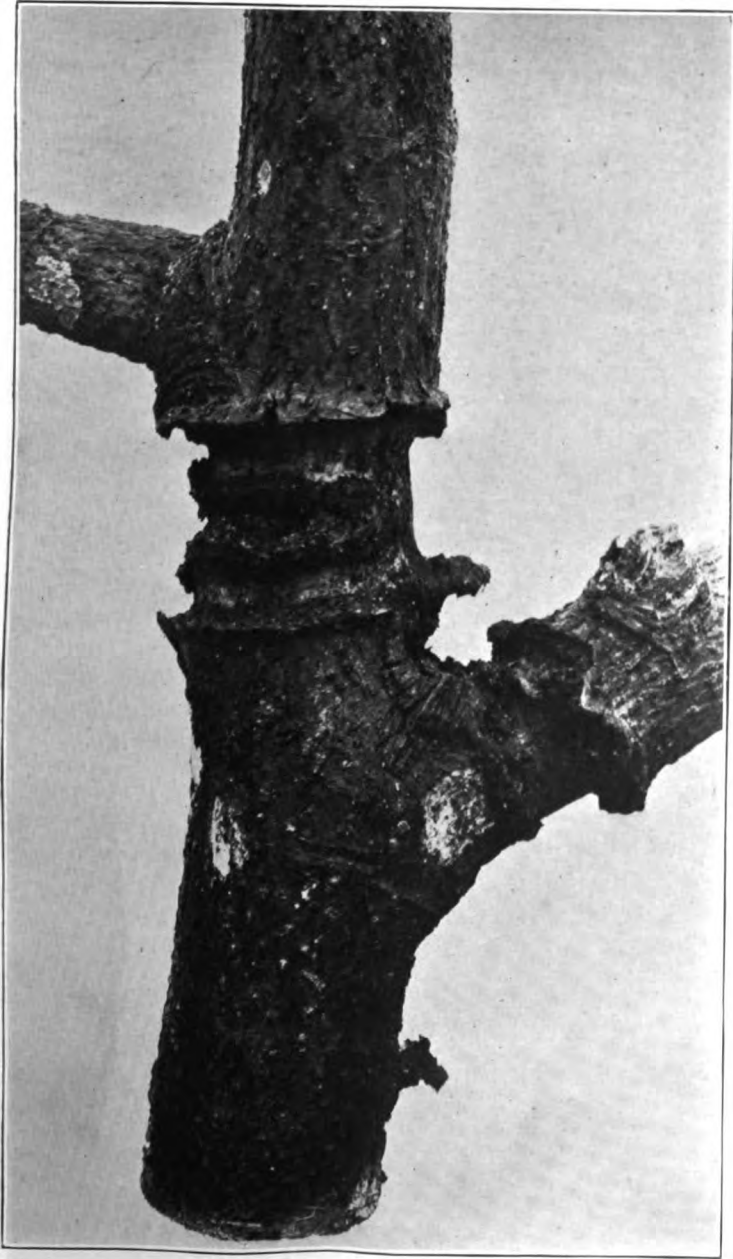


FIG. 15.



FIG. 16.



FIG. 17.

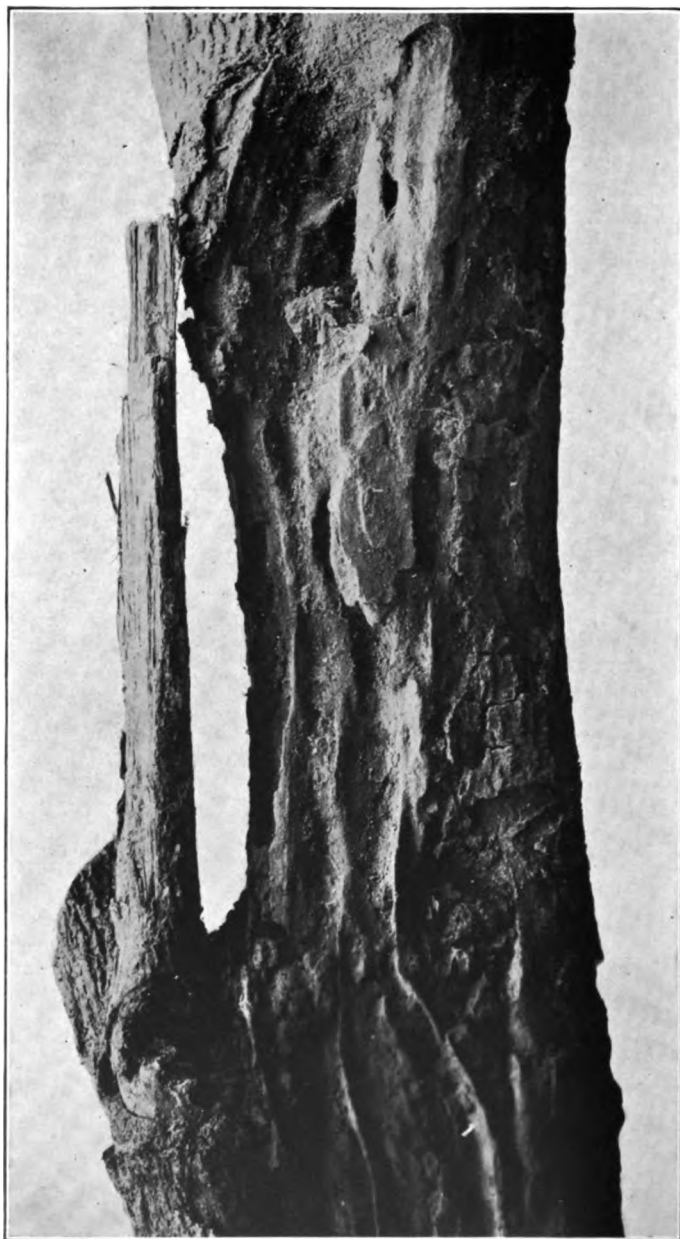


FIG. 18.

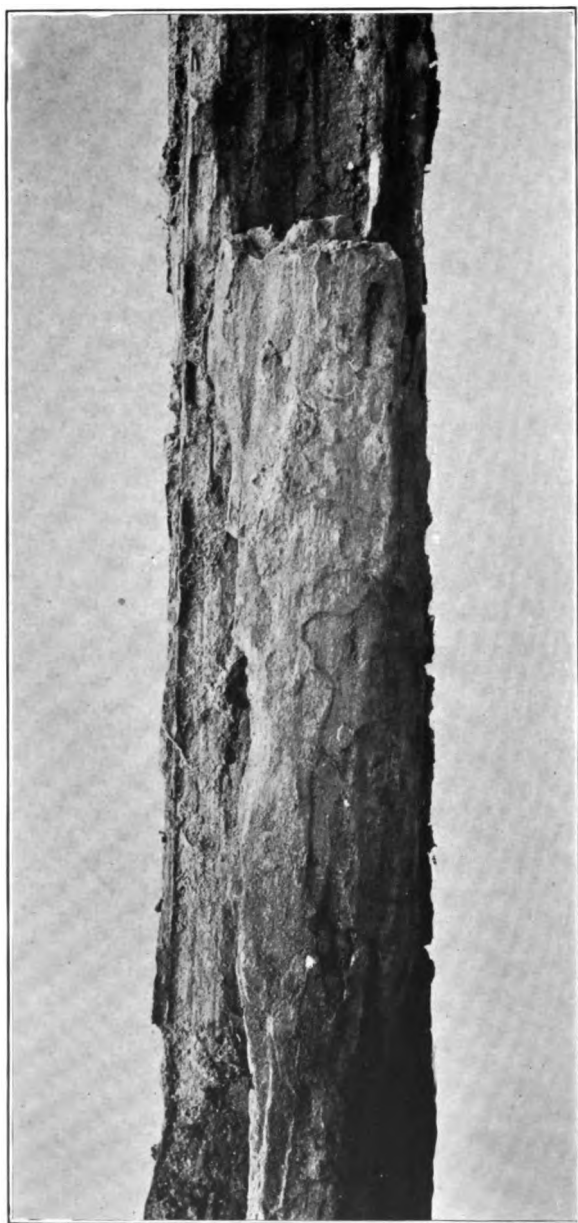


FIG. 18 a.



FIG. 18 b.

rich in food material, and thus by these successive attacks the living parts are all destroyed and the tree girdled.

Trees have been frequently observed in which, on a branch from 4 to 7 cm. diameter, there would be a strip of only 15 mm. of living wood, and yet this branch would be giving forth leaves above the wound. Of course the latter were small, being ill nourished, and no fruit could be expected upon such a branch. This illustrates the remarkable vitality of the cacao, and probably also explains its extreme susceptibility to injuries of any kind.

An interesting feature of the work of the flat-headed borers is the manner in which the excrement is packed behind it in the burrow, sometimes so solidly as to simulate the wood itself. It can, however, always be distinguished by its granular appearance instead of the fibrous structure of the wood. These filled burrows are frequently encountered in timber when it is sawed for building and other purposes. Wood having this appearance is called "wormy." Characteristic burrows of one of these larvæ are shown in figs. 18 and 18 *a*, and in fig. 18 *b* the frass as left behind.

When the grub is about to pupate it burrows toward the heart of the tree and upward, thus forming an oblique canal, which, when a short way in, is changed to a vertical direction and is thus parallel with the wood fiber. Into this burrow the insect retires and changes its position so that the head is toward the opening. Within a very short time after the larva has entered this retreat and has packed in the fibers to close up its doorway, it sheds its skin as a grub and assumes a form which, upon closer examination, shows that it possesses characteristics of a beetle. The legs, antennæ, wings, and wing-covers may all be readily distinguished if a careful examination be made. Unfortunately, there have not yet been sufficient observations upon this insect to enable me to state the length of time during which it remains in the larval and pupal stages. On March 11, 1903, a full-grown beetle was taken from a tree near one in which larval and pupal forms had been found at an earlier date of the same year. This would seem to indicate that the period for the pupa is from four to six weeks, possibly not so long.

The adult, after its outer covering has become thoroughly dry and hard, subsequent to its change from the pupa, begins the work of gnawing away the material which, as a larva, it had packed in the mouth of its burrow, and it comes forth a very beautiful creature, entirely unlike the uncomely grub which had been doing

the damage. Its length is 25.5 mm., its greatest breadth 6.5 mm. The antennae in the specimen before me measure 44 mm. When the insect is at rest, the antennae are carried back over the body, projecting beyond the tip of the abdomen. The antenna has 11 joints, the second from the head being much thicker than the others and having transverse corrugations or lines upon its upper surface. Each succeeding joint, except the three last, is knobbed at its extremity and bears a spine on the hinder edge. The eyes, which are black and composed of what appear to be a number of fine jet beads, are crescent-shaped and are placed around the hinder edges of the sockets of the antennae. The thorax is very markedly corrugated transversely and is covered with fine golden-brown hairs which give it and the wing-covers, which are similarly marked, the appearance of a beautiful brocade velvet. The legs are rather long and slender. They are covered with fine hairs, as is true, also, of the under surface of the body. The feet are provided with a pair of hooked claws which aid the insect in clinging to the bark. In the act of egg laying the female grasps the bark firmly with all her feet and is thereby enabled to insert the ovipositor into the bark.

The beetles of this family make a peculiar noise when disturbed or captured. It is similar to the sound produced by rubbing the finger nail over a very fine file, and is made by the friction of the tip of the wing-covers on the surface of the abdomen.

The question of combating this insect is a very serious one, as the methods employed must at once be easy of application and likewise effectual at the time when the insect is likely to begin its greatest damage. The beetle almost invariably rests upon the bark, which is so nearly its own color as to make it very difficult to detect. It is therefore evident that any attempts to thus discover it and rid the trees of this pest by hand picking would prove practically useless. In order, therefore, that the insect be repelled from the bark, something must be used which will prove at once distasteful to it and harmless to the tree. Probably the very best method with young trees, when recently set and until they reach an age when the bark will not be susceptible to the effects of any strong wash which may be applied, is the complete enveloping of the trunk of the tree by means of protectors made of a coarse grade of abacá or *sinamay*, such as is used in the Visayas for mosquito netting, and which has a mesh sufficiently fine to



FIG. 19.

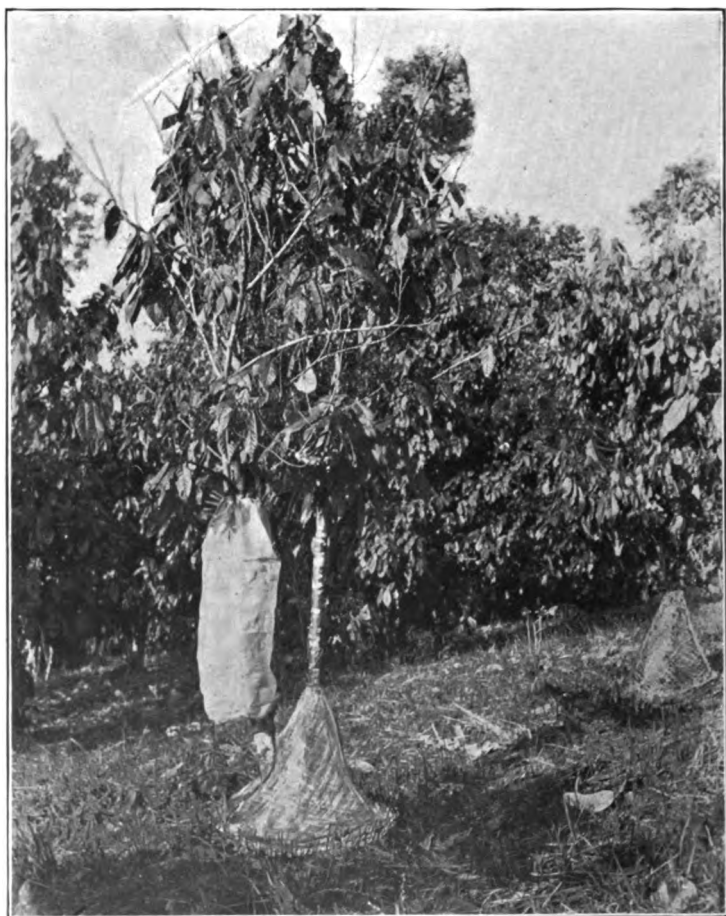


FIG. 20.

prevent the entering of the adult for egg laying. This material is so cheap that it could be used constantly, even until the trees have attained a considerable size. The bags are made of a cylindrical form, open at both ends, and having a drawstring in one end. They may be slipped over small plants and the lower ends held close to the ground, either by embedding in earth or by the placing of stones upon the lower edge. In the case of larger trees, where it is impracticable to slip this bag over all the branches, it is well to simply use a straight piece of cloth, putting it around the tree and after doubling the edges together in the form of a hem, they may be either sewed with coarse stitches or pinned by means of sticks of bamboo made in the form of toothpicks. See fig. 19 for an illustration of a bag which was used for the protection of the upper part of the trunk in order that, during investigations, the adults which emerged from the tree might not escape. In fig. 20 are shown trees protected by these bags. Opened at the bottom and held close to the ground by stones or earth, they serve admirably for keeping off the beetles. Wire screening would serve equally as well were it not for the fact that the extreme dampness would cause it to rust and thus quickly become useless. In the United States a heavy grade of tarred or roofing paper serves the same purpose, but its cost would be against its general use in the Philippines.

Where it is not practicable to use the method described above, some repellent to the insects, should be applied to those parts of the trunk which are most likely to be affected. Inasmuch as these repellents are simply supposed to keep the adult insect from laying her eggs, it is very obvious that they should be applied before any insect has had a chance to deposit its eggs in the tree trunk. If the grub has once entered the bark, no application of remedies externally will have the least effect upon it and other means must be taken. These will be spoken of later. Any strong-smelling substance, such as fish oil, tar, or pitch, which will adhere to the tree, or pure crude petroleum, would prove effectual, but perhaps the best and most easily applied material for warding off the attacks of this borer is what is known as the soap and carbolic-acid wash. This is prepared by dissolving four liters of soft soap in four liters of hot water and adding one-half liter of crude carbolic acid. This mixture should stand for at least twenty-four hours, or until it has become perfectly dissolved. Into this should

be poured from 32 to 40 liters of rainwater and the whole carefully stirred until thoroughly uniform. It may then be used with a broad paint brush or swab on the end of a stick, covering all the parts of the trunk and branches which might be supposed to offer a place for the borer to lay its eggs. No fear need be had that this mixture will injure the trees if properly prepared in the proportions given. The period from the first of April to the first of June would be the best in which to apply this preventive, as it is during this time that the beetles come forth and are ready to lay their eggs.

An excellent preventive which has been used most successfully against the peach borer in the United States and which would probably prove of value equal to that of the carbolic wash, is a preparation composed of lime, coal tar, and whale-oil soap. This mixture may be put on in a comparatively thin coating, as ordinary rains do not easily wash it from the trees. It must be thoroughly applied to all parts of the tree likely to be attacked by the borers.

Tobacco dust has been advocated by some fruit growers in America as a preventive of the borers. The author leaves this matter until more thorough experimentation in the Philippines shall determine the advisability of recommending it for general use. If it be found an effectual remedy, there would be a decided advantage in employing it in lieu of insecticides which would have to be brought from abroad.

An effective measure to be used against the grubs which have already entered the tree is to carefully search for evidence of their existence beneath the bark, and when they are located to thrust in a stout piece of bent wire, which which they can be crushed. It is not necessary to extract the crushed insect, as when so injured it can not transform further and there will be no danger of its coming forth as a beetle.

The fact must be constantly borne in mind by those who would grow cacao successfully in these Islands that vigilance, not only over those charged with the work of caring for the trees, but also personal inspection of the orchards themselves by the owners, will be necessary to detect not only the presence of these insects, but also a large number of other forms whose insidious working in places in which it is not easy to get at them, renders them all the more pernicious. As in a clean house or in a clean city, diseases are less liable to enter and play havoc, so, in a clean cacao orchard, insects

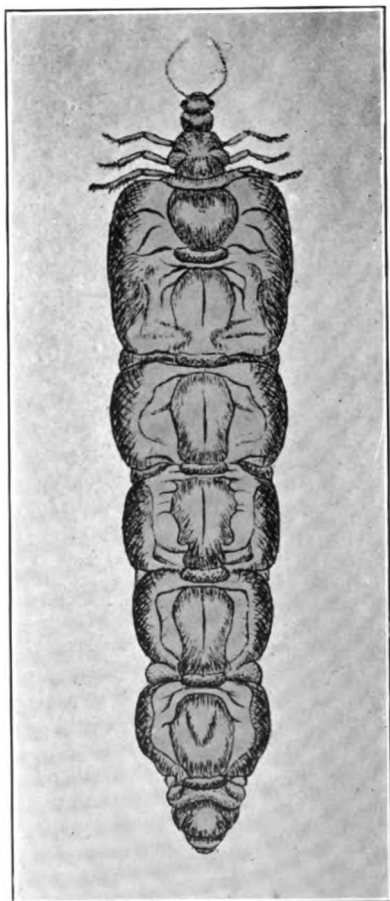


FIG. 21.

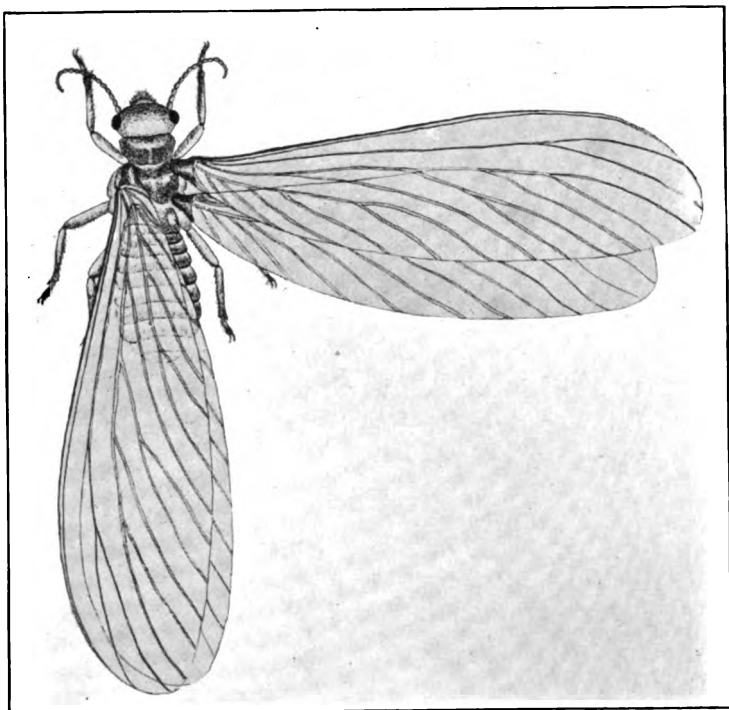


FIG. 22.

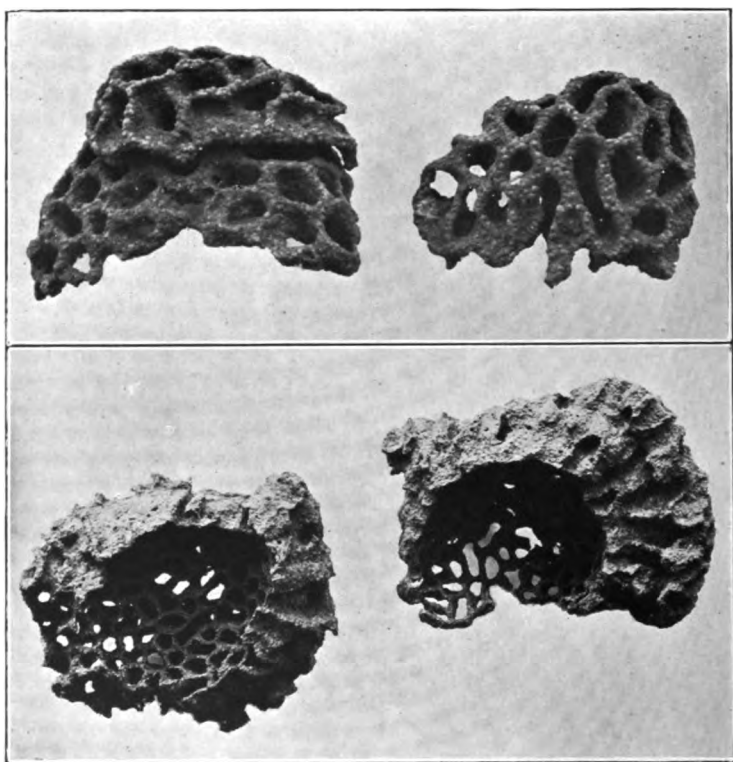


FIG. 23.

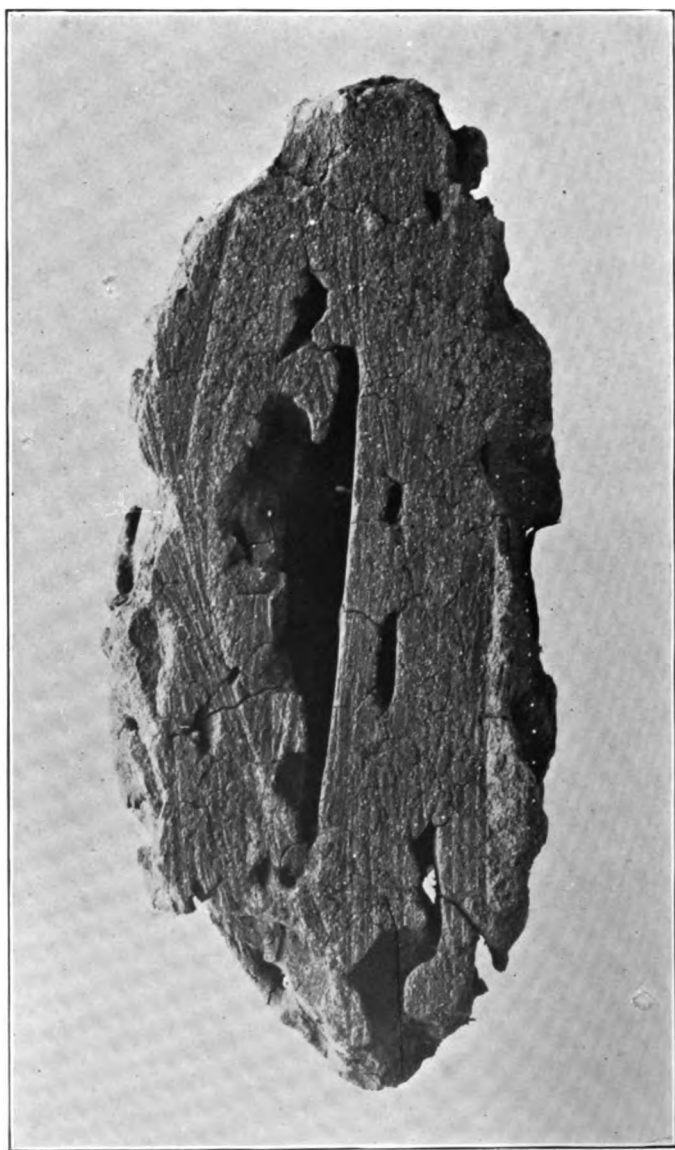


FIG. 24.

find less favorable fields for their ravages. This means that thorough and scientific pruning, careful cultivation as set forth in Lyon's Bulletin on Cacao Culture,¹ and a constant care must be taken against insect and other pests. Clean ground around the cacao trees proves less inviting to all kinds of insects which would be liable to lurk in the rubbish and dead leaves which often litter the plantations so far observed.

WHITE ANT OR TERMITE.

Another insect which has proven seriously destructive to the trunks of cacao trees is the "white ant" or termite. This insect, described as being destructive to the roots, does not by any means confine itself to these parts of the tree. Fig. 2 shows the work done in the living trunk of a cacao which had first been seriously injured by the borer. When once the white ant becomes abundant in a tree, the life of the tree is practically doomed, unless the measures suggested on page 16 be followed before the insects have extended high into the tree. These termites have an underground cell in which the queen-mother of the colony is confined; owing to her great size she can not leave this cell, and performs no other function save the laying of eggs, with which her enormous body is distended. Fig. 21 shows a female, natural size, of a species closely related to the one which damages cacao trees. Fig. 22 shows the same kind of insect before her body has become distended with eggs. Unless this queen-mother be destroyed in some way the colony will go on multiplying indefinitely, it matters not how many of the ants found in the tree be killed. There are always thousands of workers in the ground chambers of the nest to attend to the rearing of the enormous brood. Fig. 23 shows the cells in which the young are reared.

In order to find out approximately the number of eggs laid by a female, a complete queen cell in which the queen-mother was confined, was dug out. Fig. 24 shows the appearance of a cross section of this cell. The female was carefully watched for several hours and was seen to be continuously laying eggs. These eggs were carried away in tiny adherent masses by the workers. A watch was made for one minute, and instead of allowing the workers to remove the eggs during this period, the mass was carefully picked up with a fine forceps as fast as it had assumed the size of those

¹See Bul. 2, Philippine Bureau of Agriculture, 1902.

previously carried away by the workers. A count revealed the fact that during one minute 165 eggs had been laid by the queen-mother. No exact estimate of the weight of this mass was made, but it is safe to say that it represented not more than 1-1000 of that of the female when fully distended. Some idea can be gained from this of the wonderfully prolific character of the termite, and the futility of attempting to destroy a colony without first killing the queen-mother.

The same species of large black ants, which were mentioned as attacking the tree below the crown and among the roots, will likewise be found in the trunk, especially after the attacks of the borer and the termite or *anay*. They do not attack the trunk very high up, seeming to prefer living half beneath the ground. The same treatment recommended for this insect when found among the roots will apply to it when found in the trunk of the tree.

PSOCIDÆ.

A little insect which is often rather abundant upon the trunks of cacao trees is a species of *Psocidæ*. This little fellow is perfectly harmless, feeding only upon the lichen growths which are to be met with. It should not be confused with the winged forms of plant lice which are very injurious. This species of *Psocus* is red and black; the body being red and the wings mottled and of a smoky color. They are often found bunched together in considerable numbers on the lower trunk near the ground, and when disturbed scurry to one side like a flock of sheep, leaving a bare spot where the disturbing influence touched them. Among these aggregations the larvæ, which are wingless, the pupæ, which have tiny wingpads, and the adults, which have fully developed wings, may be found. Fig. 25 shows a portion of a cacao tree trunk covered with these insects. As they feed upon substances entirely foreign to the life of the tree and simply use the latter as a place of abode, they may be classified as not being harmful.

Very frequently caterpillars will be found resting upon the trunks of cacao trees during the day, while at night they will be found feeding upon the leaves. These are often so nearly of the same color as the bark that they escape all except an eye trained to look for such things. On particular species of caterpillar belonging to the family *Lymantriidæ* is so nearly like the caterpillar of *Por-thetria* (*Ocneria*) *dispar*, the Gypsy Moth, as to indicate a very

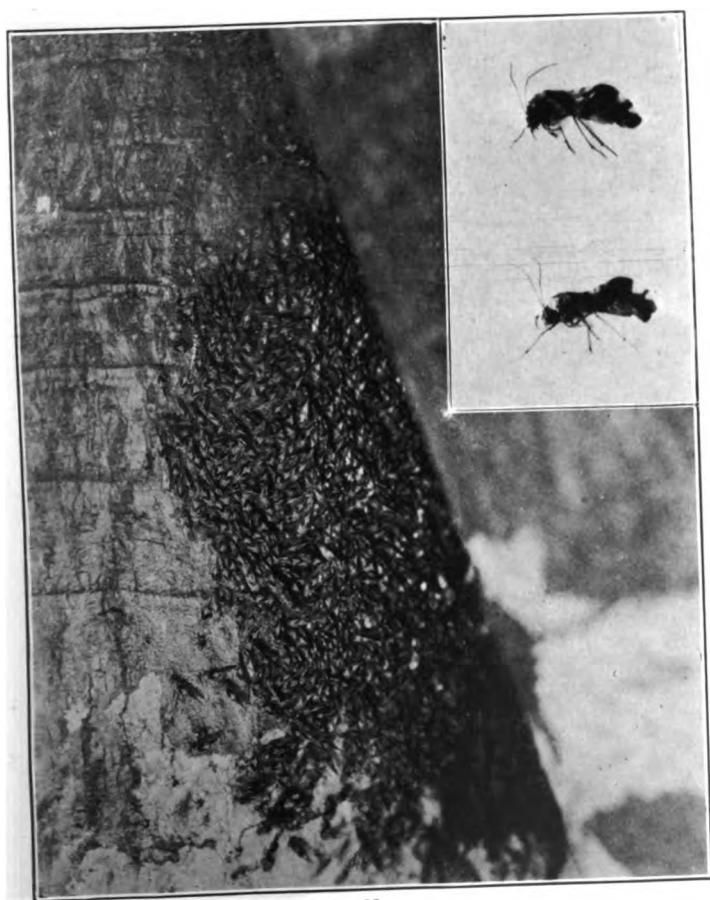


FIG. 25.



FIG. 26.

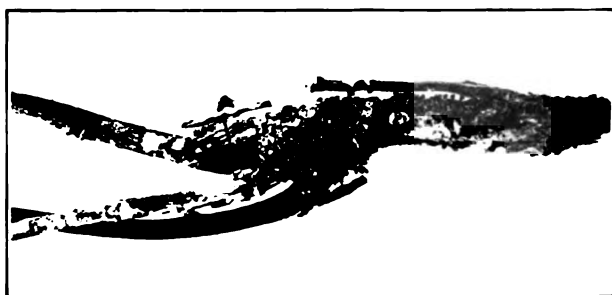


FIG. 26A.

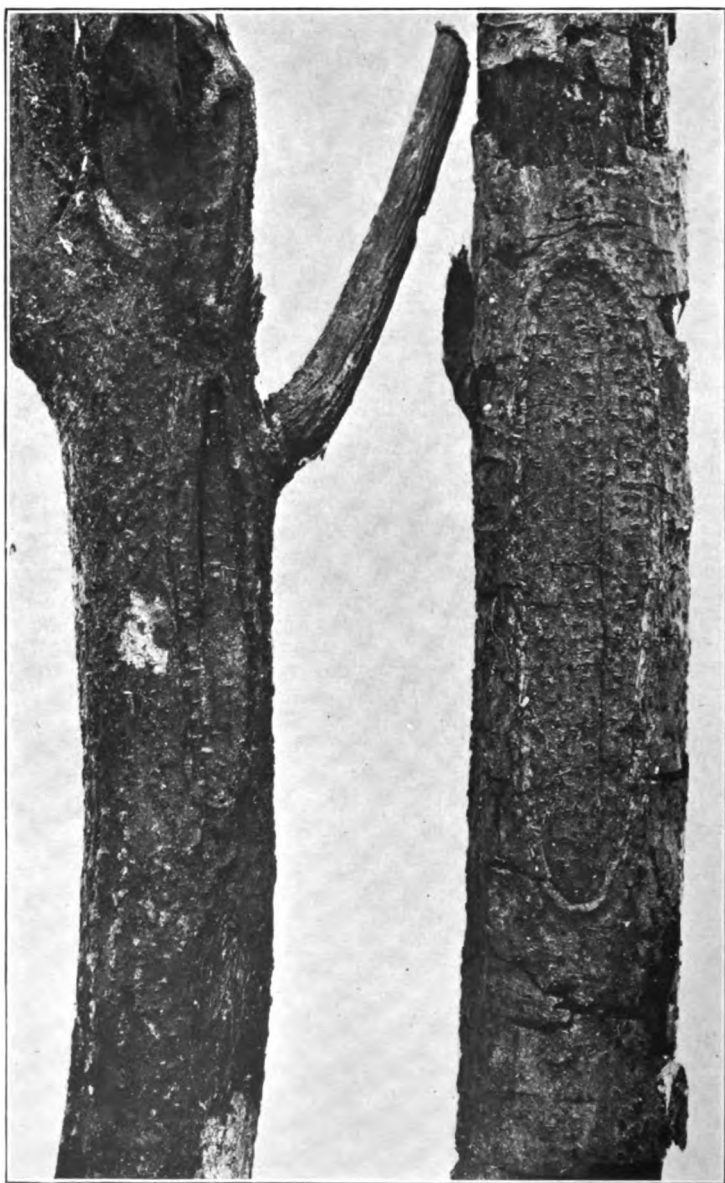


FIG. 27.

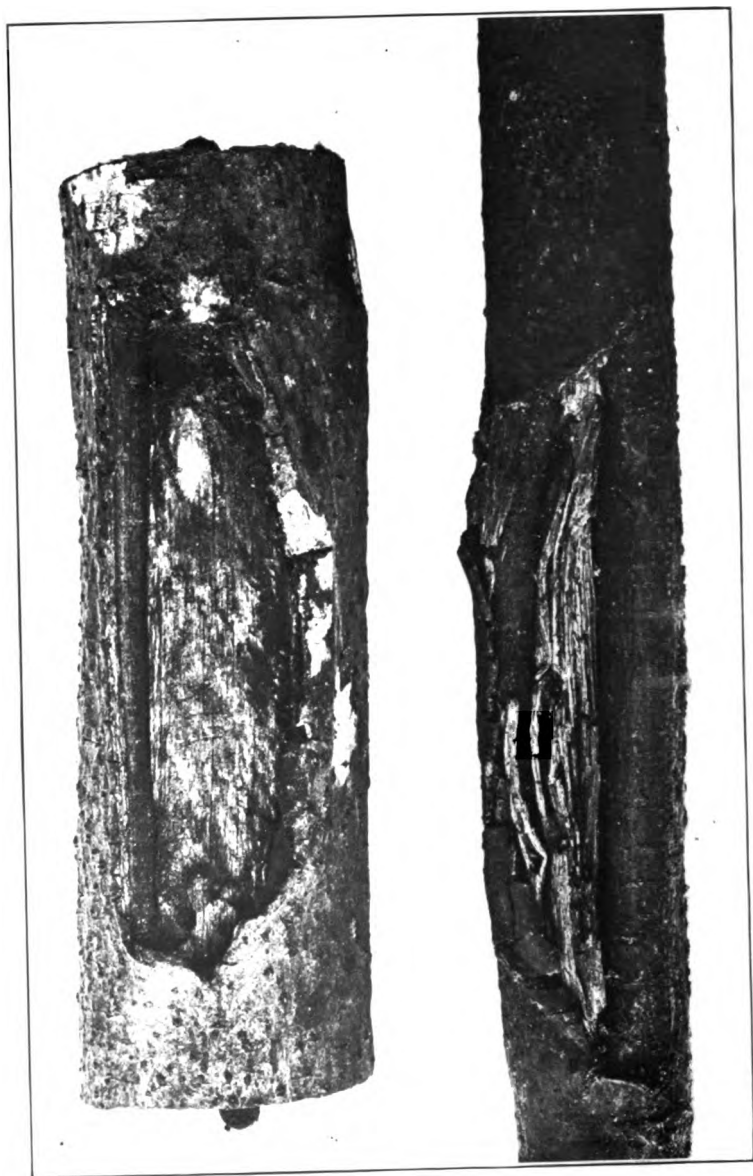


FIG. 28.

close relationship between the two, but as I have not yet succeeded in rearing the adult, the insect has not been determined. The habit of placing the cocoon or nearly naked brown chrysalis in forks of the twigs and in holes in the bark is almost identical with that of *Porthetria*.

The caterpillar will be described when the insects which injure the leaves are discussed.

INSECTS ATTACKING BRANCHES AND TWIGS.

Three very serious insect pests which attack the small branches and twigs, girdling the bark of the latter at its junction with the stem, have been found in the larval stage, but all attempts at rearing or finding the adults have so far proven failures. (Fig. 26 will show how thoroughly the larva of one has done its work of girdling a twig which was 26 mm. in diameter. Fig 26 a shows a twig 13 mm. thick which is also completely girdled by the insect.)

A peculiar and interesting habit of the larva, which first attacks the twigs to girdle them, is that of using its excrement, together with silk, for covering up the burrow or retreat in which it lives. This is very neatly done by fastening the particles together with a kind of silk and these pellets or particles of frass are so nearly the color of the bark from which the animal has obtained its food that the difference between this and the sound bark is often not noticed except upon closest observation. This larva is of a dull greyish color with a brownish head. Its body is sparsely covered with stiff bristles and its general habits are similar to those of the *Tortricida*.

The second larva is undoubtedly a *Cerambycid*, but as has been said it has only been found in this stage and can therefore not be identified at present.

The destructive work of *Cicada* has already been mentioned in reference to insects which affect the roots of the cacao and its habits of laying its eggs in the twigs make it likewise one of the insects injurious to the twigs and branches. The mode of treating twigs thus affected would be to cut off and burn all twigs in which the *Cicada* eggs have been laid. One will frequently come upon a twig or even a good-sized branch which bears a peculiar scar like those shown in fig. 27. If a careful examination be made this will be seen to contain shrivelled egg shells about 4 mm. in length. Twigs will be found like that represented in fig. 28, in which the

eggs show very plainly. The adult which lays these eggs has not yet been found, but from the general appearance of them it is very likely that the insect is an *Orthopteron*; that is, belonging to the order of grasshoppers, crickets, etc., and may be one of the katydids, some very large species of which have already been taken in cacao groves.

Microcentrum retinervis, the angular-winged katydid of the Southern United States, lays its eggs, which are of about the same size as those shown in fig. 29, upon the edges of leaves and upon the stems of the trees which it inhabits, and therefore it may reasonably be supposed that the destructive insect in the case of cacao is a related species.

With many insects of this and other species affecting the cacao, long periods of observation and the collection of more material will be necessary in order to become thoroughly acquainted with their entire life history. In many instances thus far only one stage, or the work alone, of the insect doing the damage, has been obtained.

INSECTS AFFECTING THE LEAVES, ETC.

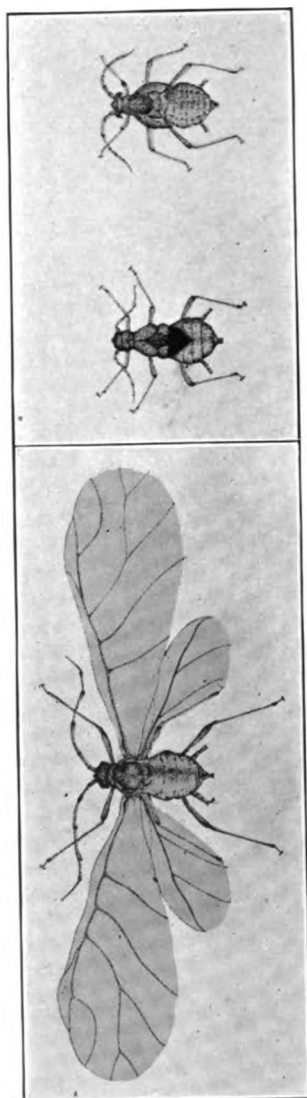
PLANT LICE.

One of the most serious pests of the leaves, tender buds, and flowers is a species of black plant louse. This insect attacks the young buds even before the leaves or the flowers have opened. The tiny eggs are laid in the folds of the buds and the bud scales, beneath the stipules of the leaves, and in the crevices of the unopened sepals of the flowers. They are so minute that they can only be seen with the aid of a magnifying glass. As soon as the young hatch, they pierce the skin of the twig upon which they rest and begin sucking the plant juices. Some broods of plant lice give birth to living young which in turn lay eggs. This question of the alteration of the mode of reproduction is very interesting to the student of biology, but has little value economically, at least in this latitude. The young plant lice resemble the adults in form. They are of course much smaller and have no wings, but as certain forms of the adults are also wingless, this feature alone will not aid in distinguishing the stages.

Plant lice are provided with a pair of spine-like projections which are little tubes on the back of the abdomen and which secrete a waxy substance which is much sought by ants. This



FIG. 29.



B

A

FIG. 30.

C

substance is commonly known as "honey dew." Its nature is not clearly known. Generally, in examining a colony of plant lice the surface upon which they rest will be found to be sticky and glistening, as though covered with sirup. This is the "honey dew" secreted by the plant lice. It is for the purpose of collecting this that ants invariably attend a colony of the former, caring for them assiduously in return for the "honey" which they secure. They frequently carry the plant lice from place to place when the leaves or twigs, upon which the latter have been feeding, become dry and hard.

Even though only a single plant louse be found upon a leaf or flower, it is almost invariably attended by an ant and sometimes by two or more. Thus cared for, it is little wonder that the plant lice multiply and flourish to a remarkable extent. Fig. 30 represents the different stages of the plant louse. It will be noticed that at *b* the pupa is distinguished by the tiny wing pads which contain the wings. These insects feed in all stages, from the larva to the adult, and therefore their damage is considerably greater in proportion than that done by insects which feed only in the larval stages, like *Lepidoptera*, *Diptera*, and *Coleoptera*. Their small size appears to be fully compensated for by their numbers, and so the cacao grower has to be ever vigilant in order that he may successfully combat them.

The immediate effect of the attacks of the plant lice is to cause a drooping or wilting of the leaves, flowers, or flower stems which they attack. This is followed by a distortion of the part, the leaves curling toward the under side, where the plant lice are usually found. This shrivelling is very marked upon some trees, and when the leaves have attained their full growth they will be found to be undersized and broken because of their efforts to outgrow the attack. Flowers, when attacked by the plant lice, shrivel and die without producing fruit. Occasionally the plant lice are found upon the very young fruits, the skin of which is almost as tender as the young leaves. Invariably fruits thus attacked either die from exhaustion, or if they survive are very much distorted, presenting, instead of the regular, even-lobed appearance of the perfect pod, a scarred smooth side which has no semblance of the characteristic ridges. In this way the pods are formed in which the tip, instead of being straight, is twisted by arrested growth on one side and by the normal development on the other.

Fig. 31 shows a fruit thus distorted, and a perfect fruit is shown in the frontispiece.

When the fruit pods have attained the size of a hen's egg they are not subject to the attacks of the plant lice and thus it will be evident that any remedy should be applied before this period.

As a means of treating plant lice upon the flowers and leaves of cacao, there is no better material than kerosene emulsion. There are several ways of making and applying kerosene emulsion, but the best preparation for this purpose will be found to be the kerosene and soap emulsion. This, if properly prepared and applied according to directions, will be found to be harmless to the most delicate parts of the plant. For its preparation see the chapter on Insecticides.

In applying the kerosene emulsion some kind of spray pump should be used to facilitate the uniform distribution of the liquid and to enable the sprayer to reach the higher branches of the tree with the emulsion. In another chapter brief descriptions will be given of some of the essentials of standard spraying machines which have been used successfully in the United States in combating insects similar to those found on the cacao.

BLACK THIRIPS.

Another enemy of the young cacao leaves which is likely to prove of considerable importance is the black thrips. This minute insect may escape even a close observer if he does not know the signs which indicate its presence. As in the case of the plant lice, the injury caused by the thrips makes the leaves curl up, as shown in fig. 32, but not as markedly as with the former. If we carefully examine a few leaves which show evidence of curlings, we will find upon the under side a number of very slender black and red objects moving very slowly from place to place. The black ones are the adults, the red ones the young thrips. The rear part of the abdomen is decidedly pointed, and the thorax bears a spine on each side. These insects are provided with sucking mouthparts and cause an injury to the leaf similar to that caused by the plant lice. They may be combated by the same means employed to destroy the latter, and large numbers of them will be killed when the trees are sprayed for the *Aphids*. Two adult thrips are represented much enlarged at fig. 33.



FIG. 31.

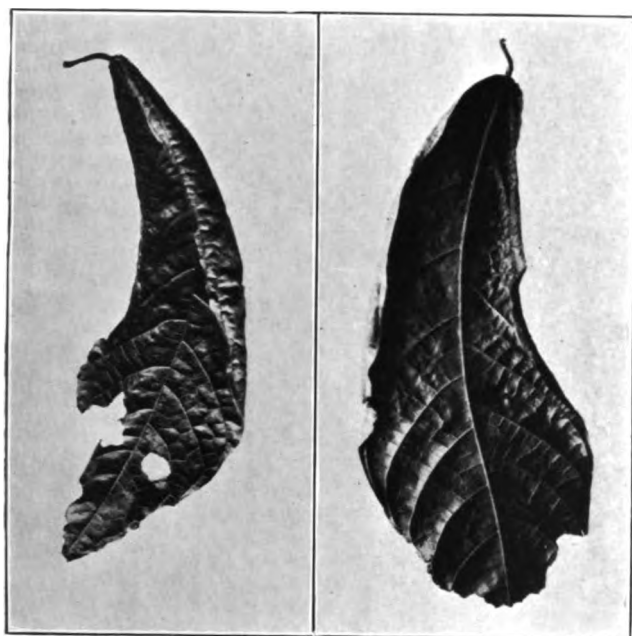


FIG. 32.

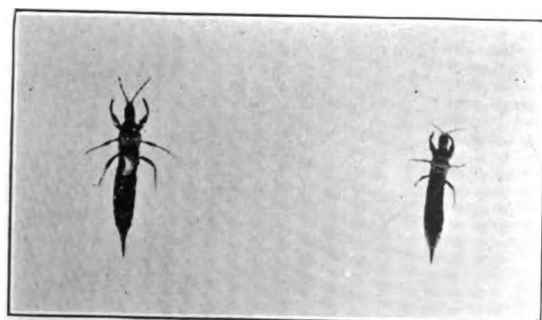


FIG. 33.



FIG. 34.

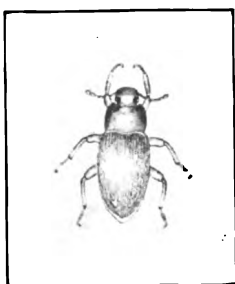


FIG. 35.

YELLOW SCALE.

Another insect which, while not occurring in great abundance at the time when observations were made, is likely to prove a pest to the cacao, is the large yellow scale represented magnified in fig. 34. Its eggs are shown at A. The adult female measures 17.5 mm. long, 13 mm. wide, and 9 mm. high. It is of a light salmon-yellow color and is covered with a fine powdery substance. Beneath, the scale secretes a white material which is fibrous in structure. The upper surface is corrugated; the hinder part of the body is much higher than the fore part, and when at rest upon the twig or leaf the black legs and antennæ are completely hidden by the shell-shaped body. The forward part of the body is slightly notched. When the animal is disturbed and moves from place to place, the tiny black antennæ and tips of the black feet may be seen protruding from beneath the shield-like body. In February and early March these insects will always be found with the space beneath the dome of the body completely filled with tiny orange-yellow eggs which are 1 mm. long and 0.6 mm. wide. It is not yet known when the young hatch, nor has anything definite been ascertained concerning the full life history of this insect. Further studies will elucidate points concerning this and other scale-like insects of the cacao.

The same method employed in ridding the trees of the *Aphids* will apply to the combating of the large yellow scale. Its soft body will make it peculiarly susceptible to the effects of kerosene emulsion.

Very frequently there will be met in the cracks and crevices under the dead bark and in holes where limbs have been broken off from the cacao a beautiful, iridescent, blackish green or blackish purple beetle with very convex wing covers and antennæ which have the appearance of a string of beads. This beetle belongs to the family *Sphindidae*, a class of beetles whose larvæ feed upon dry fungi and decaying vegetable matter and which are not injurious to the tree. When disturbed these beetles readily simulate death and will drop to the ground until the disturbance has passed. They have been found in fair abundance in all cacao plantations.

The beetle measures 14 mm. in length and 5.5 mm. in width, the wing covers being 9 mm. long. The wing covers are marked

by a series of 9 longitudinal lines which are punctuated by fine dots. The under surface of the body is of the same color as the upper surface. See fig. 35.

Occasionally there may be found upon the under surface of the beetle, tiny light-brown, roundish mites which are parasites. This is a thing which is very common to many *Coleoptera*, especially those living in dark or obscure places.

Associated with the beetle may be found several species of cockroaches, some of a very pale yellow and about 15 mm. long, others mottled buff color, 20 to 25 mm. long, and still others which are about 35 mm. long and of a reddish-brown color. None of these do damage to the trees, as they live upon the decaying matter found in the crevices and wounds.

Other harmless forms pertaining to *Thysanura*, *Coleoptera*, *Corrodentia*, and *Orthoptera* have been met with upon the trees, but a description of these minor insects and of their life histories and habits will be left for a later bulletin. In any attempt at treating the trees for injurious insects many of these will inevitably be killed.

CATERPILLARS.

The two principal destructive insects found upon the leaves are a species of caterpillar of the family *Lymantriidae* and others of the family *Euclidæ*. The caterpillar of the former, mentioned on page 27 can be easily recognized by its size and its hairy appearance. When full-grown and ready to transform it measures 33 mm. in length and 10 mm. wide, without the hairs, which add 12 mm. to these dimensions all around, making the entire caterpillar occupy a space of 53 mm. by 30 mm. on the surface of the leaf. The adult and the egg-laying habits of this insect are not known, but it is probable that the female lays her eggs in patches upon the twigs, covering them with soft down from the outside of her own body, like a closely related species found upon the ylang-ylang and the *talisay* trees. These caterpillars, as has been stated, have the habit of feeding at night and resting upon the branches and the trunk of the tree during the day. When disturbed, they raise the head and twitch the fore part of the body from side to side. If the disturbance be continued, they drop either to the ground or to a lower limb. The color is a dull gray with a few reddish markings. The head is very large, grayish, and marked by darker

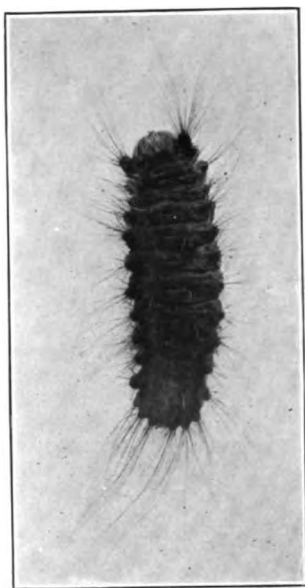


FIG. 36.

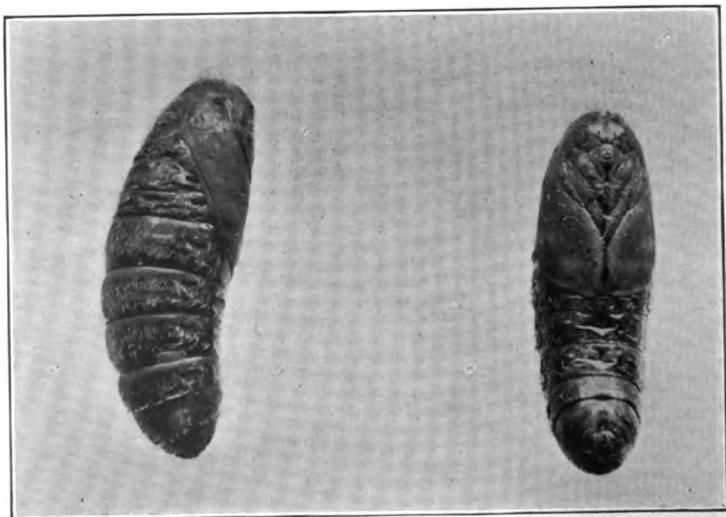


FIG. 37.

patches of small dots. The hairs which project forward from the neck give the insect the appearance of wearing a ruff. When at rest upon the bark, the false legs of the abdomen, ten in number, are spread out beyond the sides of the body, firmly grasping the surface upon which the insect is resting. When the caterpillar is about to change its skin or moult, a process which it performs five times before changing to the chrysalis, the six true feet are drawn up together, the head is drawn down toward the legs and the fore end of the body is elevated. This insect, before pupating, finds a convenient place at the fork of a small twig or in a crevice in the bark, and there it spins a very poor apology for a cocoon. The cocoon looks more like a wide-meshed basket than a true cocoon and doubtless serves simply to keep the large brown chrysalis from falling to the ground. Unfortunately, several of these chrysalids which were put into a box for rearing, transformed to the adult stage in transit to Manila from the place of capture and were so disfigured as to be unrecognizable when the box was opened.

In addition to the long hairs which cover its body, this caterpillar is provided with short, stout bristles 3 mm. long. They grow from tubercles on the upper parts of the abdominal or thoracic segments, and are spread out in tuft form. These bristles are very sharp and when the observer is pricked by them the sensation is a very painful one. It is claimed by Filipinos that these animals are very poisonous and undoubtedly this belief comes from painful experiences with their poisonous spines. On many people the prick of these spines causes great swelling and inflammation, which may continue for some days. This is due to the fact that the spines work their way through the epidermis into the lower skin and there remain for some time. If one handles these insects by mistake, the best way is to carefully pick out all the spines with a pair of pincers, using a magnifying glass if necessary to aid in the work.

The pupa is likewise covered upon certain areas with very minute spines not more than 1 mm. in length, which have the same properties as those upon the caterpillar.

The pupa of this insect is much shorter and stouter than the larva, measuring only 23 mm. in length. Fig. 36 shows the full-grown larva and fig. 37 the pupa.

SLUG CATERPILLARS.

The other caterpillar which is nearly as severe in its attacks as the hairy form is what is commonly termed a slug caterpillar. The slug caterpillar is so called from the fact that instead of possessing well-developed legs and prolegs (false abdominal legs) it has the under side of the body so modified that for its entire length it is closely applied to the surface upon which the insect crawls. The form of the caterpillar is most striking, and once recognized, these larvæ can not be mistaken for any other. The body, which in the full-grown larva measures 9 mm. long, is shaped much like a slug or snail, except that it is perfectly square across the back, the sides being also perpendicular to the back. The very pronounced margin which separates the side from the back is armed with a series of erect spines of a mottled brown color with black tips. There is also a row of these spines on each side near the ventral or lower surface of the caterpillar's body. A peculiar little tubercle armed with two of these spines projects from the rear of the body and slightly upward. When feeding, the caterpillar protrudes its head from beneath its spiny armor and when disturbed it can immediately withdraw it, pulling the fore part of the body down over it for protection. When touched upon one side the insect immediately doubles over toward that side, just as a person would do if tickled. If touched upon the opposite side, it performs a similar movement in that direction. The general color of the insect is dull brown, but the under surface is pale, almost white, and very smooth. The caterpillar secretes a kind of a slime which is evidently useful to it in its movements upon the leaf, serving in place of legs to hold it. When it is about to transform to the pupa, it spins a very tough, thick cocoon which is nearly spherical in form and is attached to the leaf or the twig. This little cocoon is brown and very smooth and glossy. It bears no resemblance to anything pertaining to an insect except to the tiny galls formed by certain hymenopterous insects upon trees like the oak, etc. It looks more like a little, dry, brown fruit of some kind.

The damage the caterpillar does to the leaves is shown by fig. 38. As the eggs are always laid upon the under side of the leaf, the insect begins by eating off the lower skin or epidermis, leaving only the veins which give the peculiar skeletonized effect shown in the figure. The insect enlarged is shown by fig. 39, while the cocoon



FIG. 28.



FIG. 39.

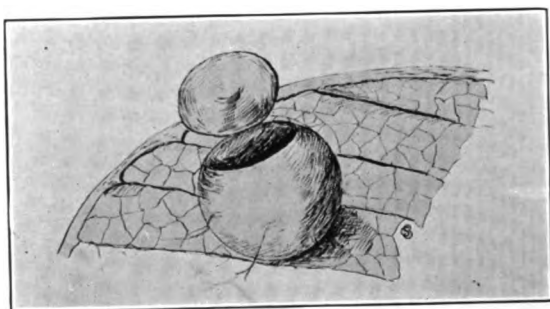


FIG. 40.

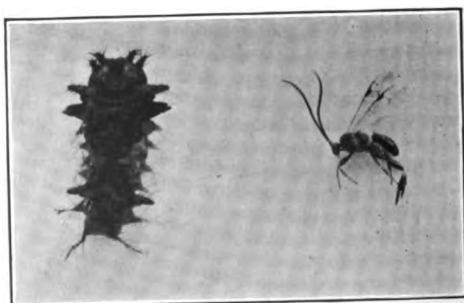


FIG. 41.

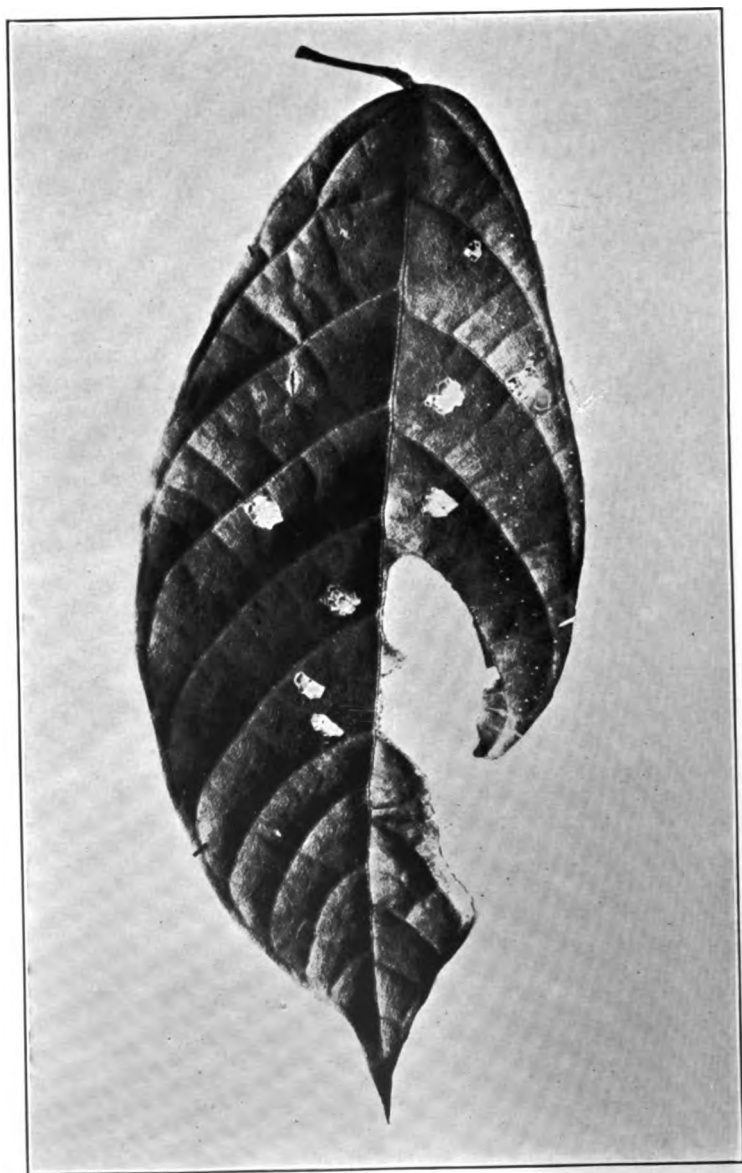


FIG. 42.



FIG. 42.

from which the adult moth has escaped is shown by fig. 40. In escaping, the moth pushes off a little round lid in the cocoon.

There is another member of the same family which, when full grown, is somewhat larger, of a bright yellowish green with a peculiar brown mark upon the back and having a double row of tubercles on either side from which grow tufts of bristles or spines very much like those described on the hairy caterpillar of the family *Lymantriidæ*. This caterpillar, shown enlarged by fig. 41, has habits similar to those of the other species of *Eucleidæ*, except that it eats holes in the leaf as shown by fig. 42.

This insect in the larval stage is attacked by a little hymenopterous parasite which lives within the body, devouring the fat and finally the internal organs of the caterpillar. When this parasite has reached the stage for transformation to the pupa, it spins its white silken cocoon within the caterpillar's body, and the latter may often be found dead upon the leaf, looking as though it were still alive but motionless.

Inasmuch as these three insects, like all caterpillars, obtain their food by biting, they will be susceptible to any poison which may be placed upon the leaf and which they can take into the mouth. Among the many insecticides prepared for this class of insects, those which are among the best are arsenate of lead, Paris green, and hellebore, the first named being probably the cheapest and most effective. When once the solution of arsenate of lead is dried upon the leaves it will withstand rain for some time before it is finally washed off, and it is doubtful if it would be all removed before the insects would have secured a sufficient quantity to kill them. As obtainable in the United States the arsenate of lead is a commercial compound, but it can be made in the quantity necessary by following the directions for its preparation in the chapter on insecticides. The preparation and use of Paris green, with other useful insecticides, will be treated in the same chapter.

Of the family *Chrysomelidæ*, there has been found at least one species which does damage to the leaves of the cacao. As this beetle is very small and of an obscure color it might be readily overlooked when searching for insects upon the plant. The female is about 4.5 mm. in length, is of an oval form and very much rounded upon the back. The male is smaller, being 3.5 mm. long. These insects vary from light to dark brown, the majority being a very dark brown. The wing covers are very glossy and are marked

by longitudinal dotted lines extending from the base to the tip. The adults are quick in their movements, both when walking and flying, taking readily to wing when disturbed or dropping quickly to the ground. They are very difficult to catch unless a bag is held beneath the leaf upon which they are resting and the latter be shaken. Even then, upon alighting in the bag they will almost immediately fly away unless promptly killed. The eggs of these insects have not been found, but as with most beetles pertaining to this family, they are probably laid upon the lower sides of the leaves in patches in which the single eggs stand on end. The young grubs are very peculiar in form, being shaped like the larvæ of ladybirds, *Coccinellidæ*, except that their abdomens are thicker at the rear. They move very slowly, and when disturbed cling to the surface rather than drop to the ground. Their bodies are covered with short black spines as a means of protection.

When young, these insects feed only upon the lower epidermis of the leaf, but as they grow larger they eat away both upper and lower skin, leaving a few of the larger veins which are too tough to be eaten. The adults as well as the larvæ are leaf feeders, eating through the entire substance of the leaf.

As many as twenty of these beetles are sometimes found upon the same leaf, and as each consumes an area equal to about ten times that occupied by its body, it can be readily seen that the damage done by the cacao leaf-beetle is considerable. For the combating of these insects a spray of arsenate of lead is recommended as fatal to both larvæ and adults.

Further observations are necessary in order to determine where these insects pupate. Most of the leaf-eaters pupate in the crevices of the bark and in dried leaves and other secluded places, such as wounds in the trees and rubbish which accumulates around the neglected ones, and doubtless those of cacao have the same habits. Fig. 43 represents the full-grown beetle, *a* being the male and *b* the female, both much enlarged. Fig. 44 shows the larva of a related species.

SCALE INSECTS.

Cacao leaves are affected by at least two species of scale insect. This is an insect which, in the larval stage, exudes a waxy secretion which forms a shell or scale which soon completely covers the individual so that none of its body is visible. This is of peculiar

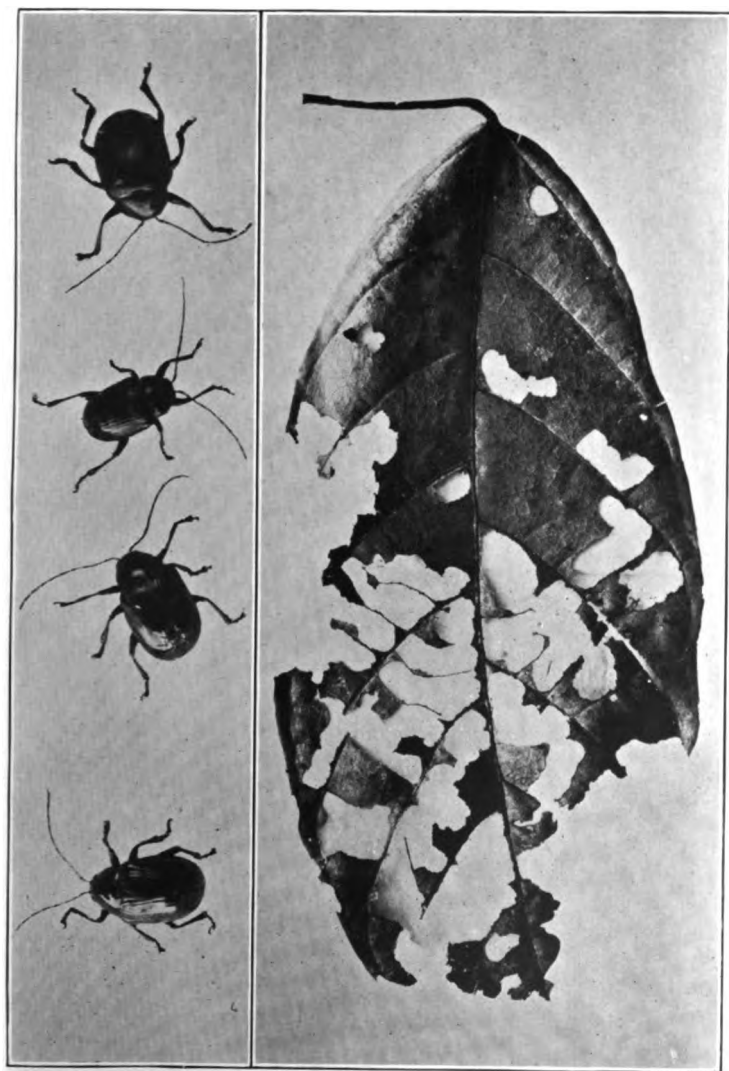


FIG. 43.

form, according to the species. Some species are long and narrow, others are elliptical, oval, or circular, others oblong. Usually there is a prominence near the center called the nipple of the scale. The scale of the adult contains the successive exuviae which have been shed by the young in its transformations. Only the adult females are found beneath the scale. They are legless, wingless, rounded masses with no eyes. Their mouth-parts consist of a very fine filament composed of three parts. This is inserted into the substance of the leaf or through the bark, and by this means the insect obtains her nourishment. Once fixed in a certain spot upon the food plant, she never moves and probably never removes her proboscis from the place where it is first inserted. She lays her eggs under the scale and the young come forth from beneath it to begin their own independent existence. The hinder end of the female's body is composed of a series of what are called anal plates. These are portions of chitine, the hard material found in the walls of all insects' bodies. They are lobed, and between the lobes are arranged series of bristles or spines. The form, number, and position of these plates are characters upon which are based the classification of these microscopic insects. Very frequently the food plant of the scale is used as an aid in its determination, but this is variable, as there are certain scales like the San José scale, *Aspidiotus perniciosus* Comst. and the oyster shell-back louse, *Mytilaspis pomorum* Bouché, which have a variety of food plants. The male larva of the insect forms a scale as does the female, but when it reaches the adult stage it comes forth a winged insect similar in appearance to the one shown by fig. 6 of the frontispiece. A young scale insect with its legs and other parts of the body complete is shown by fig. 4 of the frontispiece. At certain seasons these minute insects may be seen crawling around upon the infested leaves or twigs, but they soon settle, usually within a few days.

It is impossible to observe or attempt to study this class of insects without the aid of a fairly good hand magnifying glass. They are so small and have so little of the appearance of insects as we usually know them, that they would scarcely be taken for such by persons not familiar with the subject. For this reason they are enabled to gain a good foothold and to multiply to very great numbers unless checked by some natural enemy. In connection with the question of their multiplication it may be said that these insects, like *Aphids*, multiply with very great rapidity and

in numbers which are simply astonishing to those not familiar with their habits and life history. It is claimed that a single pair of the San José scale will in the course of a summer in the United States become the progenitors of more than three billion offspring.¹ Thus the cacao which might be slightly infested with scale at one time might very shortly thereafter be completely covered.

The first of the scale insects found upon the cacao, the *sisi* scale, so named from its resemblance to a Filipino oyster so called in Visayan, is black, shiny, and has the shape shown in fig. 45. It has thus far been found only upon the upper side of the leaf near the midrib. It is very slightly convex. Upon reversing the leaf a yellowish spot can be seen beneath the spot where the scale is lying. This is caused by injury to the leaf tissue, a result of the insertion of the insect's proboscis.

The combating of scale insects has presented one of the most serious problems which has confronted not only the grower of fruit and other trees, but also the economic entomologist, during the past few years. All kinds of remedies have been tried, such as kerosene, crude petroleum, whale-oil soap, hydrocyanic acid gas, lime, sulphur and salt wash, as well as patent articles, all of which contain some one or more of the above ingredients. Even now it is extremely difficult to say with any certainty which of these remedies, or whether any of them, will prove best adapted to the needs of the cacao grower who finds his trees threatened by this evil. No experiments have thus far been made anywhere in the Philippines with this class of insecticides with reference to the treatment of the scale insect, owing not only to the lack of the proper materials and also to lack of the machinery for their application, but also, thus far, to lack of opportunity to devote to this very interesting and important subject.

INSECTS ATTACKING THE FRUIT.

CACAO MEALY BUG.

Many plants in the Philippines, as elsewhere, are attacked upon their leaves, branches, and fruit, by several species of insects known as mealy bugs. These insects belong to the same family as the scale insects, the *Coccidæ*, and their effects upon the cacao are only second in importance to the work of the cacao borers.

¹Bul. 3, n. s., p. 44, U. S. Dept. Agr., Div. Ent.

Going through a cacao grove at the time when the trees are in fruit one notices many pods which seem to bear a whitish fungus-like material. Upon closer examination the fungus-like growth will be seen to consist of large masses of whitish insects. These insects are 3 mm. long and 2.35 mm. broad and the bodies, which are pinkish yellow, are covered with a fine powder-like substance. If examined with a magnifying glass it will be seen that this powder extends only to the sutures or joints of the segments and that the latter may be readily counted even in those individuals which have much of the powder. These bugs attack the fruit just after it has set, and multiplying thereon with great rapidity, often cover it entirely by the time it is ripe. They first begin in the depressions or grooves and gradually spread upward upon the lobes of the pod. They crowd together in large numbers and are invariably attended by swarms of ants, whose purpose is to secure the honey-dew voided by them. One of the most interesting examples of the interdependence of insects is shown in the relation of the ants to these plant lice. Upon certain pods it is a not uncommon sight to see the valleys between the lobes of the pods completely roofed over by a gray material, which upon examination proves to be a kind of crude paper, formed from particles of the decayed wood of the cacao tree. Small black ants will be seen running in and out through the openings in these sheds, and curiosity upon the part of the observer leads him to break away a portion of this material in order to find out what, if anything, is beneath it. He is rewarded by finding in the grooves thus protected, large numbers of the white mealy bugs, with their young and with willing servants, the black ants, attending and caring for them. The ants will be seen taking individual mealy bugs in their jaws and carrying them from place to place, and especially is this true of those which are exposed to view by the destruction of their roofs. The bugs submit to this with no sign of displeasure. If the observer watch long enough, he will see the ants building the roof or repairing the parts which have been broken away. They manifest great uneasiness when disturbed and all those individuals which are under the roof at the moment, come forth and assume an aggressive attitude, sitting up on four of their legs with the front ones in the air and the abdomen doubled under as if about to sting. They, however, possess no sting and only assume this attitude in instinctive imitation of those forms which do possess one. The author has found

cacao pods upon which all of the ten grooves were completely filled with the mealy bugs and roofed over by the ants. A unique feature of the fruit thus attacked was that one of the tunnels extended to the fruit stem and along the limb to the tree and then down the trunk to a spot where the ants entered a part of the tree which was decayed. Upon opening this decayed wood the true nest of the ants, with their young in all stages, was found. This would seem to indicate that the tunnels or roofs are not built solely for the benefit of the mealy bugs, but also as a protection for the ants in going back and forth from their nests to the pods where their "cows," as they are sometimes called, live. This double and somewhat intricate system shows, in a way, the wonderful provision of Nature whereby, through their inter-relationship, certain species of insects are propagated and perpetuated. It is undoubtedly true that without this provision either one or the other of the species would soon be exterminated. In as far as these animals are related to man and to his economy they must both be considered enemies, the mealy bug as being directly injurious to the product of the cacao, and the ant indirectly, as protecting and caring for the mealy bug in order to secure the product of its attack upon the tree. The latter must, therefore, share with the former in any treatment looking to its extermination.

Little is so far known of the life history of this cacao mealy bug. Its eggs have been discovered; the young have been found in great abundance in the deeper grooves of the cacao pod. In the frontispiece is shown a ripe cacao pod upon which the adult and young mealy bugs with the adult male and female, the ants which attend them, an insect parasite, and tunnels or sheds built by the ants are represented. The openings of these sheds are regularly constructed so that the ants can go in and out at certain places without disturbing the bugs. By whatever method these insects are treated, the sheds would offer but little if any hindrance to the effects of the insecticide, inasmuch as they are built of an extremely porous material through which kerosene, crude petroleum, or other similar material would soak very readily. Probably the very best solution to apply to these insects would be the kerosene emulsion recommended for plant lice on page 30. The bodies are fully as tender and delicate as those of the plant lice and therefore the same proportions of ingredients could be used. The sheds where they

are present would hold the insecticide longer in contact with the insects' bodies and would thus be a help in the extermination of the mealy bugs. Naturally the best time to spray the trees for these insects is just after the fruit has attained the size of a small hen's egg. If successfully subdued at this time the chances are that they will not reappear.

The amount of injury that these insects inflict is shown in the smaller size of the pods attacked by them, the scars which extend down into the fiber of the pods and the general inferiority in size and quality of the beans or kernels themselves. Pods infested by these bugs are not only inferior in quality to those not infested, but they are also very unsightly to those who have to handle and open them. I am thoroughly convinced that with sufficient care and the judicious use of the spray pump and the kerosene emulsion of a proper strength these minute pests may be entirely eradicated from the cacao grove, and clean, healthy, well-filled pods will be produced where now inferior ones exist.

There is another species very similar to the mealy bug, which has been found upon the cacao in a few instances. It is also quite prevalent upon the *nangka*, *Artocarpus integrifolia* Linn., being situated on the fruit stem and often on the small twigs. It is covered with a thin, white or yellowish-white incrustation which breaks very readily upon being touched, however lightly. As this insect has been found only in a few instances and on trees which were in close proximity to *nangka*, it is probable that it will not naturally attack the cacao. However, it would be well to be on the lookout for it. Further observation will reveal the facts with regard to its preference for cacao and its life history and habits may then be discussed at greater length.

BENEFICIAL INSECTS.

There are few trees which have a host of enemies without at least a proportionate number of friends in the insect world, and the cacao is no exception to this rule. In many cases the insects which in some way or another prove beneficial to the cacao are not found solely upon this tree. This does not have reference to the parasites of the insects which affect the cacao, but includes such insects as wasps, aphids, ant-lions, and spiders, the latter not being insects in the technical sense.

WASPS.

Among wasps which are found upon the cacao and which are known to feed upon larvæ and adults of noxious insects may be mentioned the *alinḡayo* and the *amomó-ong*, called here by their Visayan names. The former belongs to the true wasp family, the *Vespidæ*, and the latter more strictly to the hornets. The *alinḡayo*, which is familiar to all who live in the Visayas, because of its very sharp and severe sting and the fact that it often builds its nest in houses, is not more than 13 mm. in length. It is of a light brown color with transverse bands of yellow upon the abdomen and diagonal ones upon the thorax. The second segment of the abdomen is as long as all the other segments together and when at rest the insect retracts the hinder segments within this long one, giving it a very short, stubby appearance; but when angered and about to sting it can increase the length of the abdomen more than twice the normal size. A broad band of yellow borders the hinder edges of the second abdominal segment, which is of a deeper brown than the rest of the body. The stinging instrument is very sharp and about 2 mm. long, curving slightly downward. The eyes are slightly yellowish, the antennæ are broken or jointed at a distance from the head of about one-third of the entire length, and they droop forward in front of the eyes. When at rest, this insect folds its two pairs of wings down between the thorax and abdomen and thus they lie for a part of their length below the abdomen as shown in fig. 46 *d*. The general appearance of this insect is given in the same figure at *b*. This insect builds a nest like the one shown by fig. 47. It is often as large as a man's two hands and every cell contains a grub or a pupa. The larvæ are fat, white grubs with no sign of legs, eyes, or other appendages, and they feed upon the masticated and partly digested insects which the adult brings to them. Fig. 46 *a* shows the full-grown grub and fig. 46 *c* the pupa, which has been removed from its cell and from the delicate silken cocoon which was spun before its transformation. Soon after changing to the pupal form, the insect is a creamy white, but as the process of development goes on it assumes a darker color, beginning at the eyes, which are the first to show evidence of the true color of the perfect individual.

These insects have been seen to capture small caterpillars and

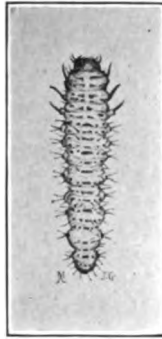


FIG. 44.

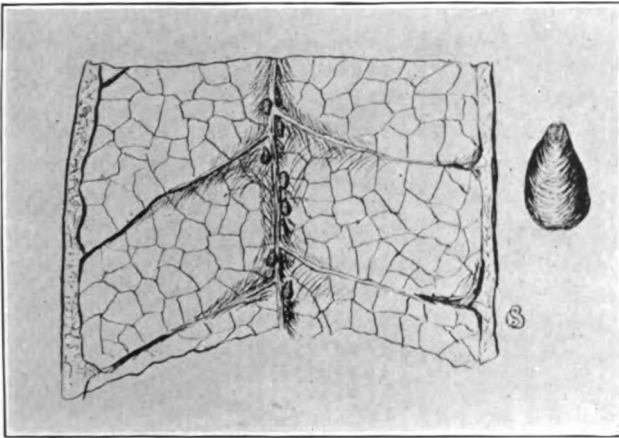


FIG. 45.

A

C

B

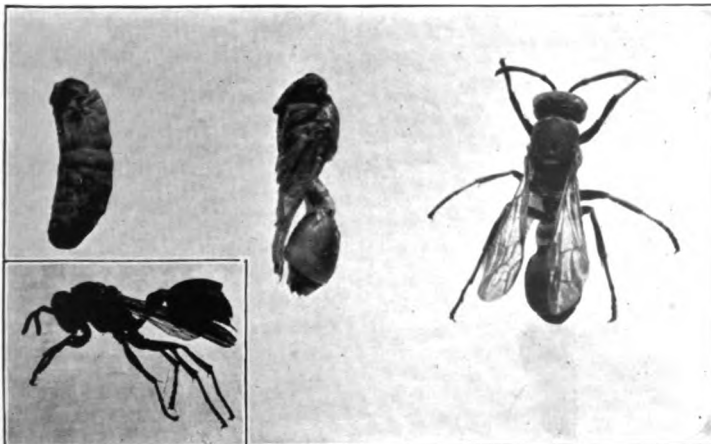


FIG. 46.

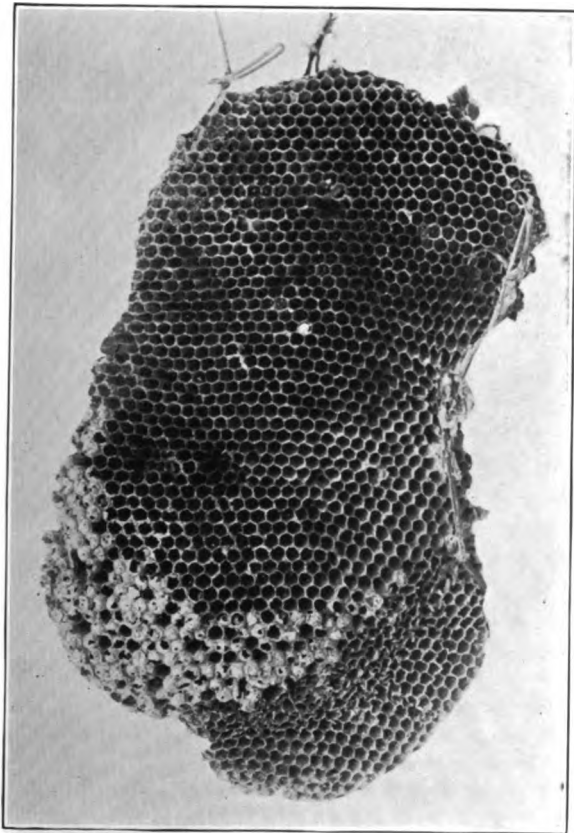


FIG. 47.



FIG. 48.



FIG. 49.

to gradually chew them into a soft mass which they carry in their jaws to their nests. They feed upon other insects and do not disdain flies. It is estimated that a colony of 100 adults will destroy in a month more than 3,000 caterpillars and other insects. Fig. 46 shows the larva, pupa, and adult of this interesting and useful little wasp.

The *amomó-ong* is very much larger than the *alinṅayo* and is one of the most beautiful wasps which I have ever seen. It is 30 mm. long and 7 mm. wide, of a jet black with three transverse bands of orange red, one on the upper part of each of the first three abdominal segments, the second being about thrice the width of the first and third. The head and thorax are of a dull velvety black. The compound eyes are black and glossy and in the top of the head are three little black glistening ocelli, or simple eyes, forming a triangle. The wings of this beautiful insect are of a reddish brown, the fore wings measuring 23 mm. in length. They are somewhat smoky. They extend beyond the tip of the abdomen. The antennæ are elbowed, as with the *alinṅayo*, and droop over the eyes. The legs and feet are of a color uniform with the body and are provided with claws for grasping the surface upon which the insect rests and for obtaining its prey. These insects have the same habits as the *alinṅayo*, but instead of building a flat nest with all the cells in the same plane, their nest has the appearance shown in fig. 49. Fig. 48 shows the adult. With this nest were captured some eight adults, about half those present, the rest escaping in my attempt to get them. These insects are valiant protectors of their homes and their young. They will remain in the nest even when struck at with poles, one or two of them darting at the intruder. When the nest has been torn away they will even come back to the same spot and attempt to build another in its place. This is contrary to the usual habit among insects, of giving up a place when once their nest is damaged or destroyed. That these insects render a valuable service in the destruction of larvæ of injurious species is beyond question of doubt. They have been repeatedly caught when returning to their nests with full grown larvæ in their claws. They carry their prey in their claws rather than in their jaws, as is the case with the *alinṅayo*, and they do not masticate it until they reach their nests.

Among the species of caterpillars which have been taken away

from these wasps are those of *Geometridæ*, *Lymantriidæ*, *Cetonia*, and *Tortricidæ* in large numbers. In all cases where they are not so numerous as to prove annoying by stinging unsuspecting workers among the trees, they should be left alone in their nests.

RED COSSAIR.

Another insect which is frequently met with upon the cacao tree and which might be mistaken for its enemy is one of the true bugs, a Reduviid which I propose to call the "*red corsair*." This *Hemipteron* belongs to a class of insects which are very properly called assassin-bugs, because their instinct is directed to the killing of other insects. The *red corsair*, a picture of which is shown by fig. 50, is a very showy insect, having a black underbody with red thorax and red wings. The head and feet are black, as are the antennæ. The insect measures 17 mm. long and 5 mm. wide. The head, which is very long and narrow, has a peculiar beak which curves under toward the thorax. This beak is very sharp and is used for sucking the blood of insects which the *red corsair* captures. At *a* is shown a dorsal view of the insect, while at *b* it is shown enlarged twice with the beak visible. The nature of the beak can be readily seen from the drawing.

This insect moves very stealthily about upon the surface of the leaf and pounces upon whatever insect it may find. It has been observed most frequently feeding upon geometrid caterpillars which it holds between its fore-feet while sucking their blood. When not seeking its prey, it rests quietly with its legs spread far apart and the body suspended above the surface upon which it is standing.

If caught in the fingers this insect is liable to inflict a sting with its beak which, while not poisonous, is very painful to say the least, and with some persons the result may be a disagreeably sore swelling. They do not, however, need to be handled and the good they do on the trees in the matter of destroying noxious insects is of incalculable value when compared with the occasional bites they may inflict on those who carelessly manipulate them. Their eggs are laid in crevices of the bark and when the young come forth they look very much like the adults, save that they have no wings. The young also feed upon small larvæ and other insects. Stål in his "*Hemiptera Insularum Philippinarum*," calls this insect *Sphodronyttus erythropterus* Burm., var. *conrivicus* Stål.

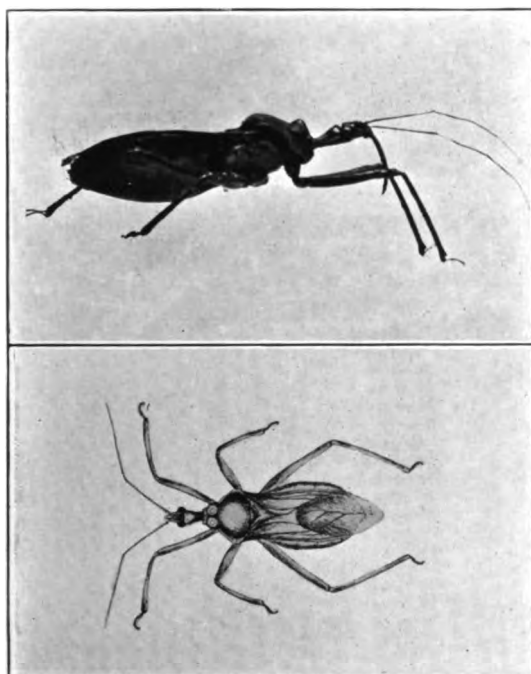


FIG. 50, a and b.

ANT-LION.

In a cacao plantation in which the top soil is sandy and in which there has been no rain for some time, one will very frequently see around the bases of the trees a number of cone shaped pits with the sides, which are composed of loose sand thrown at an angle of about 45° to the surface of the ground, coming to a point below. If an ant or a fly be tied to a string and let down into the bottom of this pit it will be at once seized by a pair of jaws which is lying concealed in the sand. If the observer be quick enough he may succeed in jerking out a peculiar-looking grey insect resembling a large-sized louse, and in fact so called by the Visayans, who name it *cotú-cotú*, meaning a big louse. This animal which measures, when fully grown, 12 mm. long and 5 mm. wide, is the larva of a very delicate and beautiful insect of the family *Myrmeleonidae* of the order *Neuroptera*. The adult measures about 29 mm. in length and has an expanse of wings of about 60 mm. The wings are iridescent and the general color of the insect's body is a smoky grey. The eyes are rather large and spherical, being prominently set, like those of the damsel flies, relatives of the dragon flies; or, as they are called in Visayan, *tumbuc-tumbuc*. One would never associate this delicate insect with the voracious *cotú-cotú*.

The larva has habits which are as strange and incongruous as its appearance. Its two forcep-like jaws are well adapted to sucking the blood of unfortunate insects which drop into the pit which it has dug for them. It invariably moves backward instead of forward. If dropped upon a sandy spot it almost instantly disappears. It uses its tail as a sort of shovel to dig its way into the ground.

Its method of constructing its pit is interesting in the extreme. After working its way below the surface it begins to move around in a circle, the diameter of which is in proportion to the size of the insect and consequently in proportion to the hole which it will finally have excavated. When it has completed its circle, it begins throwing out the sand with its head, which serves as a shovel. As the sand is thrown out the larva gradually moves toward the center of the pit which, from the force of the caving sand on the sides, begins to assume a conical shape. When the ant-lion, for such it is called in English, encounters a pebble or small stone it immediately lifts it upon its opened jaws and attempts to throw it out. It can throw out a stone which weighs much more than its body.

When the pit is completed the ant-lion goes into the sand at the apex of the cone-shaped hole, and with only its jaws and its eyes protruding, awaits the coming of some unwary insect. It usually has not long to wait, for as a rule ants and such crawlers are very curious when they come to a hole. They invariably like to investigate, and peering over the edge of this pit they lose their balance and begin to slide down just as a boy would if playing at the top of a sandhill. Their downward motion is very much accelerated by the ant-lion, which immediately begins to throw sand up over them. This takes the sand away from under their feet, and in a shower of the material they are gradually forced down into the pit. Finally, reaching the bottom completely exhausted, they are seized upon by the skillful foe, dragged under the sand, and their blood is sucked out. Afterwards their dried skins are flung out of the pit by the ant-lion.

Fig. 51 shows two views of the ant-lion's nest, one being sectional, with the insect seen at the bottom. Fig. 51 *a* shows the larva.

The ant-lion will not attack large insects such as beetles or big spiders which occasionally fall into its pit, but remains quiet until the intruder can get out. It will attack the termite, any kind of ant, small flies and bugs which fall in, and it has been seen to drag small moths under the sand. When ready to pupate, the ant-lion spins a spherical cocoon of grains of sand fastened together by a beautiful, pearly white silk, and lined with this material.

This insect can certainly be called a decidedly beneficial one, for of all the insects which it has ever been seen to capture not a single one was other than noxious, and the number of ants and *anay* which it catches and kills in course of its somewhat lengthy larval stage must be great indeed.

PRAYING MANTIS.

To the *orthopterous* family *Mantidæ* belong several very peculiar-looking animals which from time immemorial have received the attention of even the most careless observers. They are called mantis, devil's riding-horse, camel-horse, mule killer, etc., in English, and *tagâ-tagâ* in Visayan. In Tagalog they are known as *sa-sambâ*. Unlike most *Orthoptera*, they are carnivorous, living upon other insects, and are therefore to be classed as beneficial.

They lay their eggs upon the twigs of the cacao and other plants.

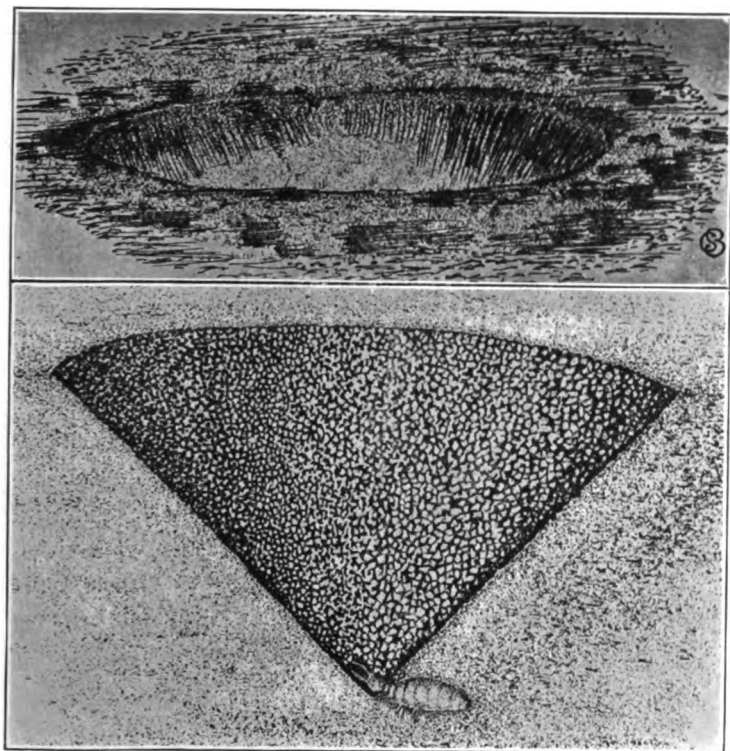


FIG. 51.

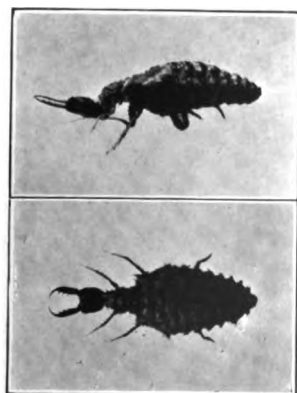


FIG. 51 a.

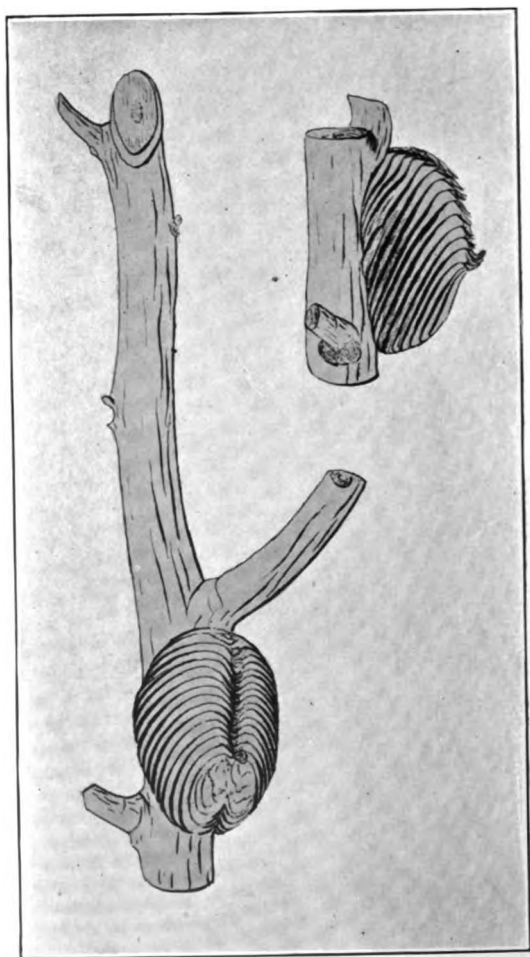


FIG. 52.



FIG. 53.



FIG. 54.

and as many as 200 of the young hatch from an egg mass such as shown by fig. 52. The young are very much like the adults in appearance. Fig. 53 shows a young mantis and fig. 54 an adult. The forelegs are fitted for grasping the prey. These insects often remain motionless upon a twig for several hours, but if an insect comes within reach, they immediately, with great rapidity, grasp it in their forefeet and proceed to eat it, only rejecting the harder parts and the wings. After feeding they very neatly clean their faces, antennæ, and front feet and again begin their watch. They run readily and are also good fliers. They rest entirely upon the two hinder pairs of feet. They have the habit of sitting up and watching the observer, who in turn becomes the observed. The large eyes and peculiarly shaped head, together with the large body, make them objects of very grotesque appearance.

SPIDERS.

The many kinds of spiders which live upon the cacao and which undoubtedly do it a service by the number of insects which they capture and devour, should be the subject of special attention, although they do not come under the technical classification of insects.

Among those which have been found most abundant are the orb weavers, the jumpers, one species of tarantula, and many species of the crab spider, or *Thomisidae*. Perhaps the most peculiar and one of the most beautiful of the orb weavers is the crescent spider, which is illustrated by fig. 55. It has its abdomen shaped in the form of a perfect crescent, is of a beautiful jet black and yellow, and the horns at the sides of the abdomen are pubescent at their tips. This spider builds a true orb web and catches many insects, the most of which are harmful to the plant. Among these have been noted plant lice, moths, plant bugs, and small *Coleoptera*, like *Scolytidae*, *Chrysomelidae*, and *Scarabaeidae*, all harmful species.

The crab-spiders are so called because of their resemblance to crabs in form and arrangement of the legs. They are of a light color, usually like that of the flowers which they inhabit, and they lie concealed in the clusters. They seize plant lice and other small insects as they come to the flowers. Undoubtedly they occasionally capture beneficial insects like hymenopterous parasites.

The majority of spiders found upon the cacao belong to the

jumpers or *Attidæ*. These spiders are sure to attract the attention of the observer because of their peculiarly beautiful and varied colorings and their activity. They are light green, golden yellow, brown and red, black, grey, and of many other and striking colors. They are extremely active, jumping forward, or backward, or sidewise with equal celerity, and they invariably capture their prey by pouncing upon it. Their eyes glow with what appears to be an internal fire, and the position on the head and the form suggest in a most remarkable way the headlights on an automobile.

A most peculiar and common form of the *Attidæ* is the ant-like jumper. These insects vary in length from 3 to 10 mm., according to the species, and their resemblance to black ants is so very great, not only in their form but in their movements, that they often deceive the close observer. They run upon only six legs, carrying the two fore legs high in the air and moving them with the same uncertain motions that ants have when using their antennæ. They will run a short way, then stop and wave their front feet, then start off sidewise, then back, and before one knows what they will do next, have pounced upon some unsuspecting fly or bug. In form and movements they really represent one of the most striking cases of mimicry. Fig. 56 shows an example of the ordinary jumping spider and the ank-like form.

At the base of the cacao one frequently sees a hole 15-20 mm. in diameter, carefully and neatly lined with snowy white silk which sometimes extends out upon the ground for some little distance. If one will dig out this hole, following its twists and turns, at the inner end, which is usually 40 or 50 cm. in the ground and parallel with the surface, if the ground be level, or horizontal if the hole be in the side of a hill or a mound around the tree, he will be almost sure to find a most beautiful dark brown hairy spider of the tarantula family, *Theraphosidæ*. This spider measures when extended, including legs, 85 mm. in length and 65 mm. in breadth. Its brown velvety coat appears almost black when it is seen curled up against the white background of its silken cell. If one of these spiders be examined when dead, it will be seen to have two very strong, curved, brown forcep-like mandibles which, instead of approximating point to point as in most spiders, are bent forward and downward so that the tarantula can not really grasp its prey with its jaws. It is, however, provided with two foot-like palpi, which serve to hold its food while it is eating. These palpi give the

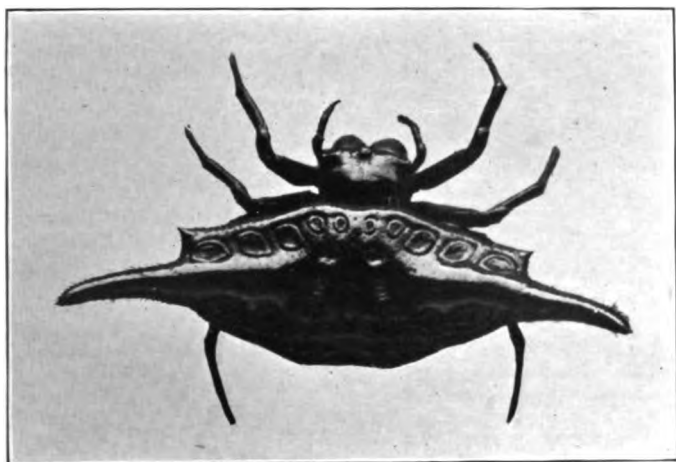


FIG. 55.

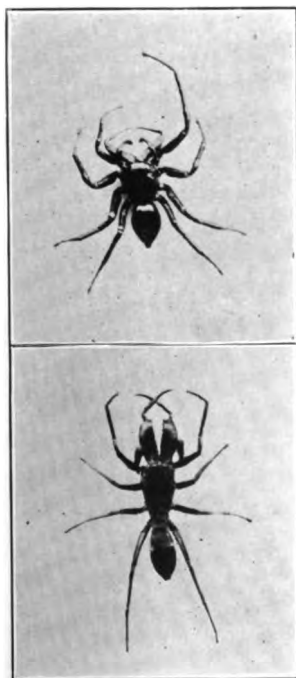


FIG. 56.

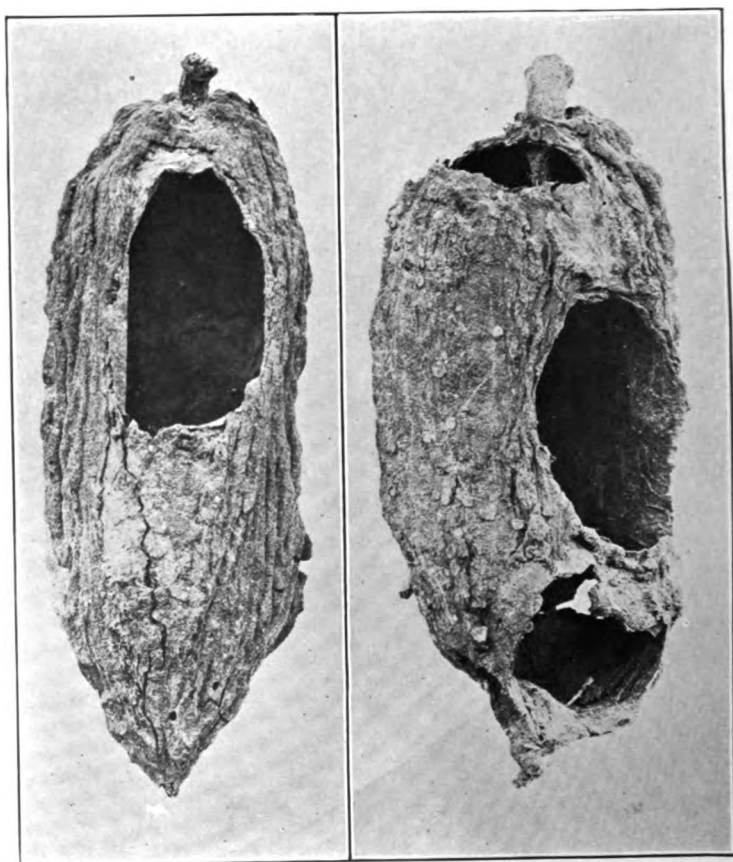


FIG. 57.

tarantula the appearance of having 10 legs instead of the true the number, which all spiders have, namely, eight.

The area around the mouth is covered with golden reddish hairs. This characteristic seems to be common to members of the tarantula family. The spider feeds upon most kinds of insects; the exuviae of various larvæ and pupæ being found in some of the nests opened. It seems to show a great preference for cockroaches, skeletons of several kinds having been found in its abode.

PESTS OTHER THAN INSECTS.

A word should be said before concluding this paper regarding some other animal pests of the cacao. In all parts of the world great trouble has always been experienced in cacao growing because of the ravages of rats and mice. These rodents, working at night when vigilance is relaxed, often do an incalculable damage to the fruits, destroying many pounds of the nuts in a season. Fig. 57 shows two cacao pods, each with a hole in the side just large enough for the small rodent to enter. The intruder very skillfully removes all the kernels from a pod and leaves it hanging on the tree, where it dries, if not removed by the grower.

No effective remedy has been devised for this pest, but the writer would suggest that an excellent one can be found in the discarded pods after the ripe nuts have been removed. The pulp on the inner side of the pod being scraped out and mixed with some kind of effective poison like arsenic or paris green, is replaced in small quantities in each pod. The pods are then to be fastened together with two bamboo toothpicks and the decoys placed beneath the trees, two or three under each one. It is more than likely that the rats, finding these upon the ground, will attack them first, and, gnawing into them, will eat the sweet pulp within. The results will then remain to be seen, but certainly the chances of success are very much in favor of the growers. All hogs and other domestic animals should be excluded from plantations in which these experiments are being tried and the refuse should be collected as soon as it is seen that the pods have been gnawed into by the rats, and it should be buried at a sufficient depth to insure its not being dug up by hogs, etc. As the pods are liable to shrivel and open during dry weather, they should be renewed from time to time as occasion will require.

INSECTS ATTACKING DRIED CACAO.

Very little can be said at present concerning insects attacking the dried product of the cacao, namely, the beans and husks. In visiting various wholesale establishments for the handling of cacao in Manila, beans have been found giving evidence of the work of some form of the common dried-fruit moths, but thus far neither the larvæ nor the adults have been discovered.

Chittenden, in his bulletin on "Some little-known insects affecting stored vegetable products,"¹ says the following in connection with a moth which he calls the "chocolate" moth. I would, however, suggest that instead of being called the "chocolate" moth it be called the cacao-bean moth, as it is more frequently found in the bean than in the manufactured chocolate:

The habits of our flour and meal feeding phycitids, *Ephestia kuehniella* and *Plodia interpunctella*, are so well known as to necessitate no further comment here, but there is still a fourth moth which, although represented in our faunal lists, seems never to have received mention as an injurious species in this country. I refer to *Ephestia elutella* Hbn. Its habits have been known in Europe since early in the last century, yet so far as I know at present, American records show nothing positive regarding injuries.

Réaumur's account of the moth that injures chocolate, published in 1737, is generally conceded to refer to the present species, and as it is this species that is most often associated with the chocolate nut of commerce it may be called the chocolate moth. Recent study of bred material shows this to be the moth mentioned in Insect Life (Vol. IV, p. 332) as having been received at this office from Mr. H. F. Wickham, who found it injurious to cayenne pepper in one of the drug houses at Iowa City, Iowa. We have also specimens bred from dried apples obtained from a New York City dealer and submitted to this office by the Division of Chemistry, and others from cacao beans received from Mr. C. A. Barber, who obtained them from Montserrat, West Indies. According to various European authorities, this species also attacks manufactured chocolate, coffee and various dried fruits, and even does considerable damage to ship biscuit, which it injures after the manner of *E. kuehniella*.

It may prove that the above-mentioned species is the one which has been found in the cacao in Manila, and the possibility is more strongly emphasized by the fact that the condition of the beans, the class of holes made, and the frass contained within the beans all point to the work of a phycitid.

Certain beetles of the families *Tenebrionidæ*, *cadelles*, or dark-

¹ Bul. No. 8, n. s., p. 9, U. S. Dept. Agr., Div. Ent.

ling beetles, and the *Nitidulidæ* or sap beetles, are also found in stored cacao products as well as in decaying or over-ripe fruits and on wounds in trees, the sap of which they suck.

For these insects, as for all insects which are found in stored products, it is difficult to suggest an effective preventive, inasmuch as they find such ready means of entrance through the meshes of the sacks. Even where the adult insects can not themselves get into the bags and sacks, instinct seems to lead them to lay their eggs upon the outside, and the tiny larva, upon hatching, find easy ingress. Once inside, the insect passes through its transformations, but upon becoming adult it can not escape and continues to multiply.

Frequent handling of the contained cacao and a careful shaking of the bags, afterwards placing them in bright sunshine, renders the cacao less liable to be attacked by any form of insect which would eat the dried nut.

Where prevention has failed, or where infested cacao is received by the dealer, the best plan is to submit the full sacks, or better, if possible, the exposed contents, to the fumes of carbon bisulphide in a closed bin or other absolutely tight receptacle. If an air-tight room can be had, the sacks may be left in it for two or three days or even longer without any harm being done to the beans, and with the perfect assurance that all insects contained therein will be destroyed at the end of that time. The bin or room should then be opened and thoroughly aired and the cacao spread out in a draught or in the sunlight.

Under no conditions should a lamp or other light be permitted in a room where the carbon bisulphide is being used, nor should persons remain in the room. If a bin be used, the bisulphide may be put on top of the cacao in a small dish, allowing 30-40 grams for every cubic meter of space. As the bisulphide is heavier than the air and very volatile, it will evaporate quickly and settle downward in the bin, suffocating all insects therein. If a room be used the vessel may be put upon a high shelf or upon some object so that it will be as near the ceiling as possible. In this way the entire room will be fumigated.

DISEASES AFFECTING CACAO.

Although the question of plant diseases such as fungi, rust spots, mould, and rot do not belong in the province of entomological

investigation, the fact that these diseases were given some attention by me during my study of cacao insects will warrant my saying a few words about them and suggesting means for their prevention upon the leaves and fruit of this valuable plant.

There are four types of fungus attack which have been most frequently met with, namely, cacao leaf-spot, cacao leaf-blight, cacao pod-spot, and pod scab. The first of these diseases attacks the leaf in spots as suggested by the name. (See fig. 58.) These spots are irregular in shape and in an advanced stage are a dull whitish grey as though the leaf had been scorched to an ash at the point, which dries and breaks off. The two types of fungus disease which attack the pod are very characteristic and will be recognized by the growers at once, as those represented in fig. 59 and fig. 60. Both of these attack the fruit in all stages of its growth. The pod spot occurs in spots similar to that which attacks bean pods in the United States. The spots soon completely cover the affected pod and not only cause a very unsightly growth, but also materially affect the development of the fruit. Fruits which are thus affected are smaller and the beans are found to be misshapen and in some cases not developed. The history of this disease, its causes, and morphology are yet to be investigated.

The pod scab is undoubtedly the worst form of disease which attacks the cacao. It is recognized in its advanced stages by the fact that the pod becomes dry either upon one side or over its whole surface. It then cracks open in unsightly gashes as though hacked with a knife. Of course the contained beans in such pods are worthless. This is another disease of which the causes are not clearly known.

Although diversified in their modes of attack, it is undoubtedly true that the same means of prevention will apply to them all since they are fungoid or bacteriological in character. Many thorough tests covering a period of several years have demonstrated the efficiency of a mixture of copper sulphate (blue vitriol) and quicklime, commonly called Bordeaux mixture, for nearly all kinds of fungus diseases affecting plants. The blue vitriol is the fungicide or destroyer of the fungus spores, and the lime is added to keep the vitriol from burning or scorching the leaves or fruit. This solution, a formula for which will be given under "Insecticides and fungicides," will of course have to be applied to the trees by means of spray pumps and a very fine spraying nozzle.

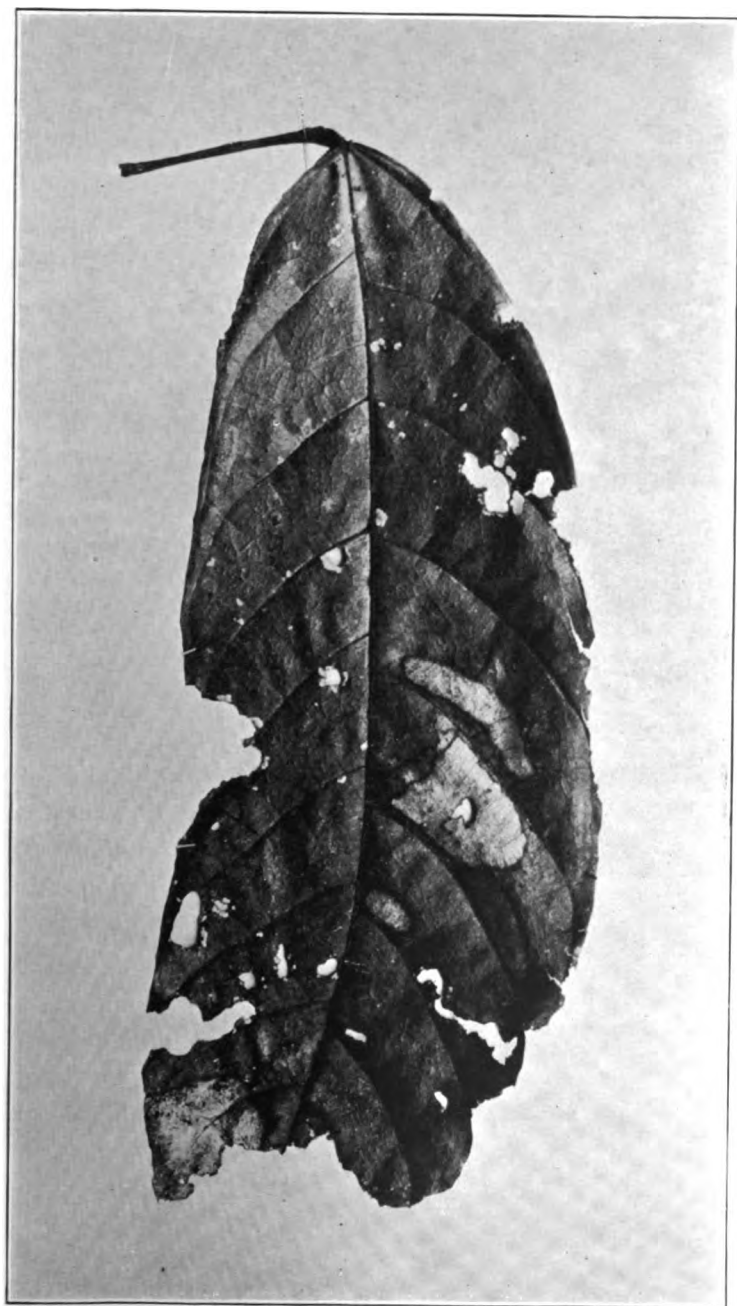


FIG. 58.



FIG. 58.

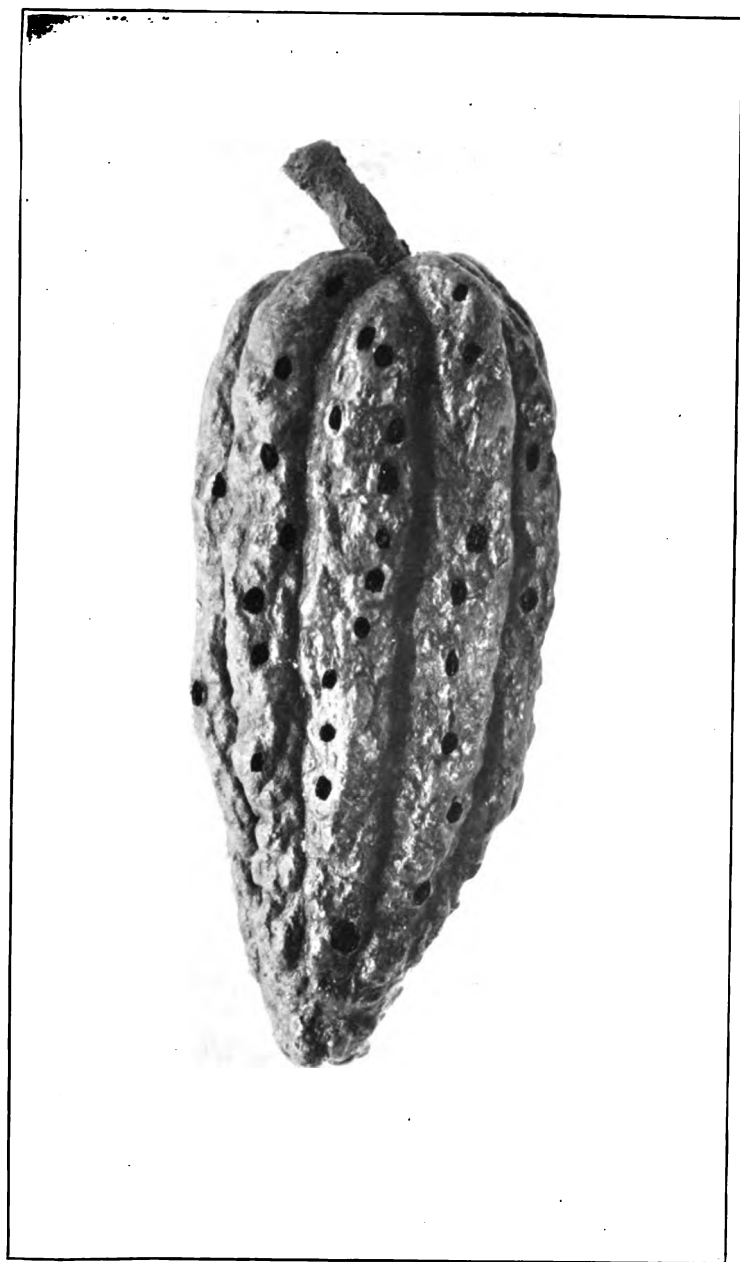


FIG. 59.



FIG. 60.

The writer is well aware of the fact that in the limited time during which he has studied the questions of insects and diseases of the cacao, many forms and conditions have escaped his notice, but as this is only a preliminary bulletin and issued so that the grower may have something at hand to guide him in the recognition of and dealing with the most important pests, the fact that all pests are not treated will not detract from its usefulness. He would be under great obligations to all growers of cacao to whom this bulletin shall come if they will send him suggestions of methods which they have tried with success.

There is a disease called "die-back" mentioned by Lyon in his bulletin on cacao, which I have not found in the Philippines, and I would be grateful for specimens showing this disease.

The production of cacao has such a favorable future in these Islands that any measures which will tend to a better degree of cultivation and a more thorough protection of trees from unnecessary ravages should be welcomed by growers and prospective growers throughout the Archipelago.

SOME INSECTICIDES AND FUNGICIDES.

THEIR USE AND PREPARATION.

Insecticides and fungicides have for their object the prevention of the attacks of insects or fungus diseases or the killing of forms already present, either upon the plant or other substance affected by the insect or the disease.

With reference to insects there are two kinds of insecticides, the internal and the contact. Of the latter there are the corrosive poisons, which destroy the substance with which they come in contact, and the suffocating poisons, which act upon the breathing apparatus, producing suffocation.

Internal poisons are those which are intended for the insects which eat the substance of the leaf or other part of the plant. Applied to the surface of the leaf either as a powder or as a solution they are taken by the insect along with its food, and, acting upon the alimentary canal and other internal organs, accomplish their work.

Contact insecticides are those which, coming in contact with the body of the insect, produce a condition, usually like that caused by a corrosive, which results in death. To this class belong ashes, lime, kerosene, carbolic acid, crude petroleum, pyrethrum, and smoke.

Suffocating poisons are those which, entering the tracheæ, either

clog them up or paralyze the muscles of respiration, thus killing the insect. To this class belong carbon bisulphide, naphthaline, bezine, petroleum, kerosene, whale-oil soap, and the fumes of hydrocyanic acid gas.

Among the most important of the internal poisons are those which have as a basis some form of arsenic. This substance in any form is deadly to all animal life. As there are many forms of arsenical insecticides, only those which are considered as of value in connection with the combating of cacao insects will be mentioned.

Arsenate of lead.—This insoluble chemical compound is prepared as follows:

	Grams.
Lead acetate.....	200
Sodium arsenate.....	50

Dissolve each ingredient in 16 liters of water separately in wooden or stone vessels. Then pour these at the same time into a barrel or tank containing 450 liters of water. The mixture should be stirred constantly and rapidly while making. Inasmuch as the compound which is formed (arsenate of lead) is insoluble it is precipitated as a white powder and must be kept in suspension. In order to make this mixture stick well on the foliage to which it is applied about two and one-half liters of glucose or grape sugar should be added to it before spraying.

A commercial compound of arsenate of lead mixed with glucose, in the form of a thick white paste, is sold in kegs in the United States, one form bearing the trade name, "disparene." This insecticide possesses all the good properties of that made at home by the grower, with the advantage that it is mixed and ready for use upon the addition of water in proper quantities. This has proven one of the most effectual and therefore most economical of insecticides against insects preying upon the foliage of fruit, shade, and ornamental plants.

Paris green.—This is a copper arsenite-acetate and contains from 55 to 60 per cent of arsenious acid. It is a bright green powder, very heavy, and hence does not stay suspended in the water of a spray pump unless continually agitated. As it does not dissolve in water it is not liable to "burn" the foliage. If, however, twice the amount of freshly slacked lime be added to a given quantity of paris green, one can be sure that all danger from free arsenious oxide will be averted and there will be no "burning" of the foliage of the most delicate plant.

Paris green would undoubtedly prove an excellent poison for mice if applied, mixed with meal or bran, and the pulp of the cacao in the manner mentioned on page 49. The proportion should be one part of the paris green to fifty parts of meal or bran.

In using paris green as a liquid insecticide it is applied as follows:

Paris green.....	grams.....	450
Lime (fresh slacked).....	do.....	900
Water.....	liters.....	1,500

This is a cheap insecticide, is in universal use, and if care is used to keep it in suspension in the water while spraying, it will give as good results as any insecticide of its class.

Numerous other arsenates are sold under the following names, and others: White arsenate, pink arsenate, white arsenoid, parine green, laurel green, London purple, paragrene, and green arsenoid.

Kerosene.—This is a refined product of petroleum which is so well known as to render a description of it unnecessary. Applied pure, to any kind of insect, it immediately destroys life. It is likewise fatal to plant life if undiluted. Used in a mechanical mixture with water, it is a remedy against scale insects and plant lice. A safer and much more effective preparation is the kerosene-soap emulsion, which is prepared as follows:

	Per cent.
Kerosene (9 liters).....	67
Water (4.5 liters).....	} 33
Common or whale-oil soap (225 grams).....	

Heat the solution of soap and water and add it boiling hot to the kerosene. Churn the mixture by means of a force pump and spray nozzle for five or ten minutes. The emulsion, if perfect, forms a cream, which thickens on cooling and should adhere without oiliness to the surface of glass. The above formula gives 13.50 liters and makes when diluted 120 liters of wash. (Report of the entomologist: In Report of the Commissioner of Agriculture [U. S.] for 1883, p. 152.) Another formula which may be of interest is the following, taken from Dr. J. A. Lintner's Second New York Report on Injurious Insects, 1885, page 38:

"Eight parts of water and one part of soft soap thoroughly amalgamated forms the lye which takes mineral oil and thoroughly mixes with whatever proportions of the oil be added. As heat aids much in quickly producing thorough amalgamation of the

ingredients, boil the soap and water together, and when ready, turn into ordinary wine bottles (costing little or nothing, especially in the Philippines) which have been placed in boiling water. About half fill the bottles, turning 125 c. c. of the oil into each bottle, then fill up with the boiling lye, cork at once and store away for use.

"When required for use a bottle of the mixture is poured into an 18-liter watering pot which is filled up with soft water, and is ready for use, at a strength of 70 c. c. to 4.5 liters of water (1 part oil to 64 parts water is about $1\frac{3}{4}$ per cent). Seventy c. c. of oil to 4.5 liters of water is strong enough to kill *aphides* (the plant lice in question) and such soft-bodied insects.

"By bottling the mixture as above, no mistake need be made in using it of proper strength."

Whale-oil soap.—A preparation made of fish oil with lye. Effective against scale insects and plant lice, and when mixed with carbolic acid makes an excellent deterrent for borers. When used as a spray for plant lice the proportions should be:

Whale-oil soap.....	grams.....	450
Water	liters.....	45-90

This should be thoroughly dissolved before using, and the weaker solution is advised for trees when in young leaf. Even 450 grams to 125 liters of water is not too weak. If ordinary soft soap or turpentine soap be used the following are the proportions:

Common soap.....	grams.....	450
Water	liters.....	18-36

Another excellent preventive which has been used most successfully against the peach borer in the United States and which would probably prove of equal value to that of the carbolic wash is what is known as the "Lime, coaltar, and whale-oil soap wash." It is prepared according to the following formula:

Unslaked lime.....	kilos.....	25
Coal tar.....	liters.....	6
Whale-oil soap.....	kilos.....	6

The lime and tar should be slaked together with water sufficient to make the whole of the consistency of paint. The whale-oil soap is dissolved in hot water and added to the lime and tar solution. When water is added sufficient to make the whole mass as thick as ordinary paint, it may be applied to the trees.

Tobacco wash.—This very effectual remedy against plant lice has certainly the advantage of cheapness in a country where tobacco is abundant. It is applied as a spray after being prepared as follows:

Dry stems or tobacco leaf.....	grams....	225
Water	liters....	4.5

Steep over a slow fire for some hours, then when ready to use it, dilute the quantity with 200 to 400 liters of water.

Carbon bisulphide.—This is a colorless liquid resembling refined kerosene in appearance. It is of a very disagreeable odor and extremely volatile. It can not be handled around fires, and even smoking while using it is dangerous. It is explosive and also takes fire very readily. Its vapor is heavier than the air, hence it should be placed above whatever it is desired to fumigate. In closed bins or in rooms the proportions to be used are: 450 grams for every 27 cubic meters, or 17 grams for every cubic meter. Whatever it is placed into should be perfectly tight in order that the fumes may not escape.

Bordeaux mixture.—This valuable fungicide has as its essential ingredient the sulphate of copper, commonly called blue vitriol, or blue stone. It is prepared as follows:

Copper sulphate or blue vitriol.....	kilos....	1.80
Quicklime (unslacked)	kilos....	2.25
Water	liters....	225

The copper sulphate should be dissolved in half the amount of water or 100 liters in a wooden vessel such as a half barrel. The lime should be slacked in the remainder of the water, and when ready for use the two solutions should be poured together into the spray-pump, barrel, or tank. In order to save time a stock solution of each substance should be made and kept on hand.

First slack in a wooden vessel 22.7 kilos of lime. This should be immediately strained into a barrel holding 225 liters and the barrel filled with water. Cover the barrel to prevent foreign matter from getting into it.

The copper sulphate stock solution should be prepared by dissolving 18.1 kilos of the crystal in 225 liters of water and putting it into a barrel, covering the same.

When it is desired to use Bordeaux, take 22.5 liters of each solution and add enough water to make 225 liters of the Bordeaux mixture.

SPRAYING APPARATUS.

At the present time the question of spraying in the Philippines is a very serious one, owing to the fact that none of the goods of large and reliable firms of spray-pump manufacturers are represented in the Philippine market. For several years past the United States Government has carried on experiments for ascertaining the best apparatus for combating insects on a large scale, and as a result of its work and the experimentation of individuals, there are now upon the American market several very excellent spray-pumps which, considering the ease of manipulation and the fact that they will last for several years if given proper care, are very reasonable in price.

The principal essentials of a good spraying apparatus are: That it deliver a fine spray, that it keep the mixture to be sprayed in constant agitation in order that the ingredients may be kept in uniform suspension, and that it have sufficient capacity so that a reasonable amount of the insecticide may be made and used at one time.

The two types of spraying apparatus which would give the best results in cacao plantations are the barrel pump, and the small power spray pump. The former would be useful in small plantations of not more than two or three hectares, and the latter for large plantations. With a barrel pump and two nozzles, and three men to operate it in the orchard, from 50 to 75 cacao trees could be sprayed in a day. With the power spray pump a proportionately larger number of trees can be sprayed. The best nozzle is one which will break the liquid up into the finest particles possible and at the same time is not liable to clog, or, if clogged, may be easily cleaned.



1903.—No. 12.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

BIOLOGICAL LABORATORY.

REPORT ON

SOME PULMONARY LESIONS PRODUCED BY THE BACILLUS OF
HEMORRHAGIC SEPTICÆMIA OF CARABAOS.

By PAUL G. WOOLLEY, M. D.

MANILA :
BUREAU OF PUBLIC PRINTING.
1904.

10885

LETTER OF TRANSMITTAL.

OFFICE OF THE SUPERINTENDENT OF LABORATORIES,

Manila, P. I., January 21, 1904.

SIR: I have the honor to transmit herewith a "Report on Some Pulmonary Lesions Produced by the Bacillus of Hemorrhagic Septicæmia of Carabaos," by Dr. Paul G. Woolley, pathologist, Biological Laboratory.

I am, very respectfully,

PAUL C. FREER,

Superintendent Government Laboratories.

HON. DEAN C. WORCESTER,

Secretary of the Interior, Manila, P. I.

LETTER OF SUBMITTAL.

BIOLOGICAL LABORATORY,

Manila, P. I., November 13, 1903.

SIR: I have the honor to submit herewith and recommend for publication a "Report on Some Pulmonary Lesions Produced by the Bacillus of Hemorrhagic Septicæmia of Carabaos," by Dr. Paul G. Woolley, pathologist, Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,

Director Biological Laboratory.

Dr. PAUL C. FREER,

Superintendent, Bureau of Government Laboratories,

Manila, P. I.

REPORT ON SOME PULMONARY LESIONS PRODUCED BY THE BACILLUS OF HEMORRHAGIC SEPTICÆMIA OF CARABAOS.

By PAUL G. WOOLLEY, M. D., *Pathologist Biological Laboratory.*

The lesions caused by the bacillus of hemorrhagic septicæmia in cattle are legion. Subcutaneous and lymphatic suppurations, gastrointestinal ulcerations and hemorrhages, widespread subcutaneous and subserous edemas, pathologic joint conditions, and varying types of pulmonary changes are frequently seen, sometimes alone, but usually accompanied by ecchymoses. During the epidemic of hemorrhagic septicæmia through which the Government carabaos have lately passed we had opportunities to study many of these.

Among the animals dead of the prevailing infection was one in whose lungs were lesions so like those of contagious peri-pneumonia that we were at some loss to make a positive diagnosis until careful pathologic and bacteriologic examinations had been made. Unfortunately the autopsy had to be done under such unfavorable circumstances and so hurriedly that there are necessarily some gaps in the protocol which can not be filled, yet the known clinical facts, together with the bacteriologic and pathologic findings, leave no room for doubt as to the nature of the disorder.

Case I. Pleuro-pneumonia.—The animal was a fairly well-nourished carabao which had arrived in Manila (from Shanghai) three days prior to coming under our observation. It had seemed well and had acted in a perfectly normal manner since landing. On the evening before death the overseer had noticed nothing peculiar about it. There was no cough and it ate and drank. The next morning the animal was found dead in its stall.

Since its arrival in Manila it had been with a herd which had come from China at the same time, and, while a few of the others had died, it was proven that the cause of death had been hemorrhagic septicæmia and they had shown no lesions similar to those found in the animal under discussion. Since its death other members of the

herd have died also, but in these there have been no lesions resembling pleuro-pneumonia.

The autopsy was done early in the afternoon of the day of death. On opening the body there was none of the subcutaneous gelatinous edema which has been so characteristic of the cases of hemorrhagic septicæmia studied by us; neither were there any extravasations of blood in the subcutaneous tissues. The abdominal cavity showed nothing remarkable, though the liver presented a number of abscesses in which flukes were found.

The remarkable lesions were in the thoracic cavity. When this was opened a quantity of pale clear amber fluid gushed out. In the residual liquid in the pleural cavities were some fibrinous shreds. The pleural surfaces were, for the most part, covered with a well-marked fibrinous exudate which could readily be peeled off, leaving a reddened, congested, roughened surface. The pleura itself was thickened and edematous. The subpleural tissues were in places filled with a sero-gelatinous exudate, and this condition was most marked under the mediastinal surfaces. The mediastinal connective tissues were completely filled with the same gelatinous material. The pericardium in its whole extent was lined with a fibrinous exudate and its surfaces were separated by a serous fluid containing flakes of fibrin.

The lungs were not collapsed, but contained air only in the anterior and apical portions. Cut surfaces of the organs were firm and red, in some places very dark, and divided by fine and coarse bands of what appeared like edematous connective tissue, so that the whole section had a marbled appearance. These bands varied from one-eighth to one-half of an inch in thickness and were in places quite saturated with serum and even honeycombed with small cystic areas filled with a bluish-looking, gelatinous material. There were no hemorrhages in the thoracic organs except in the heart. The right auricle of this organ was nearly black with large and small confluent hemorrhages. The mediastinal and pre-scapular glands were enlarged, pale, and showed areas of necrosis.

Bacteriologic.—Smears made from the heart's blood, from the liver, lungs, and pre-scapular lymph glands, showed a considerable number of small, oval, polar-staining bacilli. Cultures were made on agar, from the heart's blood and from the bands in the lungs. Within twenty-four hours small, translucent, shining, moist colonies appeared on the surfaces, resembling dewdrops. The organisms

comprising these colonies were short rods, which when stained with 1-10 carbol fuchsin or carbol thionin, showed well-marked polar staining. All the cultures made at autopsy gave the same kind of growth, and the organism was present in pure culture. The other features of this bacillus were that it did not stain by Gram's method, did not form spores, did not liquify gelatin, and did not coagulate milk. In peptone solution, after twenty-four to forty-eight hours' growth at 37° C., it gave a well-marked "cholera-red" reaction.

Intrapleural injection of small amounts of broth culture ($\frac{1}{4}$ c. c. forty-eight-hour culture) killed a guinea pig in something less than twenty hours, and a post-mortem examination of the dead animal revealed a well-marked fibrinous pleuritis, a fibrinous pericarditis, and hemorrhages into the pericardium and pleura. The lungs were partially solidified. The pancreas was surrounded by a gelatinous tissue which produced the impression that the organ had been embedded in a perfectly clear gelatin. The organism was recovered in pure culture from the heart's blood and pleural exudate, and smears from the heart, liver, kidneys, spleen, and lungs showed apparently the same organism.

Pathologic.—In sections from the lungs the air spaces contained a granular material and an occasional leucocyte or desquamated endothelial cells. The blood vessels were all intensely congested and filled with red blood cells. The mucous membrane of the bronchi was desquamated in some places, and these tubes contained a fibrino-purulent material. The bands seen at autopsy were for the most part composed of fibrin and leucocytes, but the largest of them, those extending down from the pleura, also contained a considerable amount of fibrous tissue. The smaller bands ran in all directions across the lung tissue. Such bands were rather sharply outlined from the surrounding edematous lung tissue, but they contained the hyaline, degenerating remains of the air cells which they had involved and which were filled with leucocytes. It was in these fibrino-purulent bands that the bacteria might be seen in sections stained with methylene blue and eosin, and it was from one such band that the cultures described above were obtained. Occasionally about such bands a well-marked leucocytic infiltration was observed, so that the tissue appeared like that in the gray stage of hepatization in pneumonia. In such cases the bacilli were present in the air spaces. These bands were not homogeneous. Some

were composed of nearly solid masses of leucocytes and fibrin, but many of them were formed of an external layer of polymorphous cells, leaving an intermediate clear space, free of cells, but across which fine filaments of fibrin were interlaced. Occasionally, too, other larger cells of endothelial origin were enmeshed in this fibrous lacework. Then, too, the congestion, which was general, was more intense about these bands. There were no signs of periarterial fibrosis, but on the contrary the blood vessels seemed normal save for the congestion.

The liver showed no more than well-marked congestion of the centers of the lobules.

It seems then that this may be considered as a pure case of the infectious pleuro-pneumonia, and not as one of the contagious type.

The facts in the clinical history seem to support Theobald Smith's theory of the etiology of the disease. The ocean trip, a rough passage, rough handling, all would tend to produce the primary broncho-pneumonia and emphysema upon which the later stages follow. In this case, the broncho-pneumonia was perhaps the first stage of the disease. The presence of a very infectious disease in the same herd would account for the presence of the causative organism in the lungs of the infected animal. But even without the bacilli of hemorrhagic septicæmia in other animals, the organisms might have invaded the weakened animal from the upper respiratory tract where they might have been present, and probably were, if the same conditions hold here as in the cattle which Moore examined. There is, too, a very good reason for the presence of these organisms in cattle here, if, as has been proved in other places, they are present in water. The health of the carabao, or water buffalo, depends to a great extent upon the daily bath, which is usually taken in a wallow, in the thick mud of which the animals immerse or embed themselves until only the ears, eyes, nose, and horns are visible. Frequently the whole head disappears from sight. Habits of this sort offer every inducement for such organisms as are present to enter the animal. However, we have not been able to demonstrate the bacillus of hemorrhagic septicæmia in the water or soil.

Case II.—The animal was very weak when first observed, but in fair physical condition otherwise. The conjunctivæ were somewhat congested, respiration was rapid, and the feces normal. Temperature, 40°.2 C. When taken off the truck at the Laboratory it

staggered a few steps and fell on its side. There were numerous bruises on the body, probably the result of a rough voyage across the China Sea. It ate food when placed near it and also drank, although it did not, apparently, suffer from thirst. It had no cough. During the next few days it became a little brighter and somewhat stronger. On June 6 it was again weak and could not stand up, the hind legs seeming to be especially feeble. It gradually became weaker and diarrhea developed, but with no traces of blood or mucus. Death occurred on the ninth day after landing.

The post-mortem examination showed a few patches of subcutaneous edema on the sides. There were a few small pericardial hemorrhages about the base of the heart. The lungs showed a number of subpleural nodules, which on section exposed granular areas similar to those seen in broncho-pneumonia in the stages of red and gray hepatization and suppuration. The suppurating areas were filled with a thick, granular, greenish-yellow, sticky material.

Cultures were made from the lung abscesses on agar and blood serum. After twenty-four hours at 37° C. the agar tubes showed a growth of small, transparent, grayish, round colonies. The blood serum showed a very scanty growth of small colonies. Transfers were made from these tubes to various other media, and plates were also made. After a careful study of its morphologic and cultural characteristics, it appeared that the organism under consideration was a short bacillus with rounded ends, and nonmotile. Its measurements varied between 1 and 2 microns in length, and 0.3 and 0.5 micron in thickness. The largest forms were seen in glucose media, the smallest on potato. From the animal body it showed well-marked polar staining, although this was not so distinct in organisms grown on artificial media. It was stained easily with the usual watery aniline stains, but was not stained by Gram's or Weigert's methods. The rods, as a rule, occurred singly, often in pairs, occasionally in chains of five or six individuals. The appearance of the growths on the usual culture media was in no way characteristic. The colonies on agar were small, grayish, transparent, and well circumscribed, with little or no tendency to spread. On all the solid media approximately the same appearance was noticed. In gelatin no liquefaction was caused. In bouillon a granular deposit was formed on the sides and bottom of the tube.

During the first few hours of growth the whole medium was faintly clouded, but as the sediment was deposited the liquid became clear. After a few days the sediment became viscid, as could be shown by shaking the tube, when the precipitate rose, not in floccules, but in threads. In Dunham's peptone solution the same general characteristics were observed as in broth, but the growth was not so abundant. The cholera-red reaction was produced by the addition of sulphuric acid (free from nitrates) at the end of twenty-four to thirty-six hours. No phenol was detectable. No gas was produced in solid glucose or lactose media, and the reaction of the media was not changed. Milk was unaffected even after two weeks. No acid was produced, no coagulation occurred, and there was no reduction of litmus. Stab cultures in solid media showed nothing remarkable; the growth followed the line of inoculation closely, with no tendency to spread; it extended to the bottom of the punctures, finely granular and composed of small colonies. The surface growth was small, just surrounding the point of entrance of the needle.

This organism was pathogenic for monkeys, small birds, rabbits, and guinea pigs, when injected subcutaneously, intravenously, intrapleurally, or intraperitoneally. Death was the result of a septicæmia or acute sero-purulent inflammation with subsequent septicæmia.

Sections of the lungs in this case showed a general edematous condition with well-marked congestion. There were some areas of emphysema. The peribronchial tissue was infiltrated with red blood cells and leucocytes, which filled the air spaces and which were enmeshed in a network of fibrin. Occasional bands of fibroid tissue were met with, extending down from the pleura. Some of these showed infiltration with leucocytes. There was no perivascular fibrosis.

The pleura itself was thickened, but showed no evidence of chronic inflammation. The tissue beneath it was, however, infiltrated with small round cells, and showed well-marked, new vascular formation.

Case III.—A native horse. In this case no clinical history was obtained. The lung lesions corresponded with those of Case II. There was likewise a gelatinous edema about the base of the heart. Cultures were made from the small abscesses and nodules, and in smears and cultures an organism was present that agreed in every detail with the one from Cases I and II.

The sections from the lungs showed congestion. The air cells were either widely distended or filled with a fibrino-purulent material or a granular material which resembled coagulated albumen. As a whole, the fibrous tissue in the lungs was increased. There was a considerable subpleural accumulation of well-formed granulation tissue, and there was an increase of peribronchial fibrous tissue. The bronchi were filled with fibrino-purulent material, in some cases mixed with the desquamated lining cells of those tubes. In some places, too, the lining mucous membrane of the bronchi was thickened. Extending down from the pleura into the pulmonary tissue were some considerable bands of fibrous tissue, but in this case they showed but little round-celled infiltration and no leucocytes.

The smaller consolidated areas resembled the gray stage of hepatisation. The larger ones were veritable abscesses, in the sides of which the hyaline remains of air spaces could be seen, but in the center no such remnants, but only the nuclear material and cells undergoing karyorrhexis appeared.

Therefore the cases cited above were examples of the invasion of the lungs by the bacillus of hemorrhagic septicæmia. How they gained access to the lungs we can not state with absolute certainty, but we suspect them to have come from the upper air passages and believe the pulmonary invasion was subsequent to a bronchitis in all three cases. It is tolerably certain, too, that in all of the cases death was the result of a terminal septicæmia incident to the entrance into the blood stream of the organisms which were present in the lesions of the lungs.



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1904.—No. 13.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

BIOLOGICAL LABORATORY.

**A FATAL INFECTION BY A HITHERTO UNDESCRIBED CHROMOGENIC
BACTERIUM: BACILLUS AUREUS FETIDUS.**

By MAXIMILIAN HERZOG, M. D.

MANILA:
BUREAU OF PUBLIC PRINTING.
1904.

15506

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- No. 11, 1903, *Biological Laboratory*.—Entomological Division, Bulletin No. 1, Preliminary Bulletin on Insects of the Cacao. (Prepared Especially for the Benefit of Farmers.) By Charles S. Banks, Entomologist Bureau Government Laboratories.
- No. 12, 1903, *Biological Laboratory*.—Report on Some Pulmonary Lesions Produced by the Bacillus of Hemorrhagic Septicæmia of Carabao. By Paul G. Woolley, M. D.

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1904.—No. 13.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

BIOLOGICAL LABORATORY.

**A FATAL INFECTION BY A HITHERTO UNDESCRIBED CHROMOGENIC
BACTERIUM: BACILLUS AUREUS FETIDUS.**

By MAXIMILIAN HERZOG, M. D.

**MANILA:
BUREAU OF PUBLIC PRINTING.
1904.**

16595

LETTERS OF TRANSMITTAL.

OFFICE OF THE SUPERINTENDENT OF LABORATORIES,

Manila, April 6, 1904.

SIR: I have the honor to transmit herewith a report on "A Fatal Infection by a Hitherto Undescribed Chromogenic Bacterium: *Bacillus Aureus Fœtidus*," by Dr. Maximilian Herzog, Pathologist of the Biological Laboratory.

I am, very respectfully,

PAUL C. FREER,

Superintendent of Government Laboratories.

HON. DEAN C. WORCESTER,

Secretary of the Interior, Manila, P. I.

BIOLOGICAL LABORATORY,

Manila, P. I., March 23, 1904.

SIR: I have the honor to transmit herewith and to recommend for publication a report on "A Fatal Infection by a Hitherto Undescribed Chromogenic Bacterium: *Bacillus Aureus Fœtidus*," by Dr. Maximilian Herzog, Pathologist Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,

Director Biological Laboratory.

DR. PAUL C. FREER,

Superintendent of Government Laboratories, Manila, P. I.

ILLUSTRATIONS.

- FIGURE 1. Agar tube, twenty-four hours' growth.
2. Litmus lactose agar tube, twenty-four hours' growth.
 3. Agar plate, twenty-four hours' growth.
 4. I, Glycerine agar tube, twenty-four hours' growth; II, Lactose agar tube twenty-four hours' growth; III, Glycerine agar tube, two days' growth, anærobic; IV, Salt agar tube, twenty-four hours' growth.
 5. V, Litmus lactose agar tube, twenty-four hours' growth; VI, Litmus milk tube, several days' growth; VII, Twenty per cent gelatine tube, twenty-four hours' growth; VIII, Potato tube, three days' growth.
 6. Microphotograph of a cover-glass preparation from an agar culture twenty-four hours old. Stained with dilute carbol-fuchsin. Zeiss Aplanachromatic homogenous oil immersion one-twelfth of an inch, compensation ocular No. 8. Length of bellows, thirty-four centimeters.
 7. Microphotograph of an interstitial inflammatory focus in the kidney, showing a small mass of bacilli. Magnification same as in fig. 6.
 8. Microphotograph of an interlobular inflammatory, periphlebitic focus in the liver. Zeiss objective A. A., Compensation ocular No. 8.
 9. Microphotograph of a degenerated glomerulus, almost complete fibrosis. Magnification same as in Fig. 8.



Fig 1



Fig 2

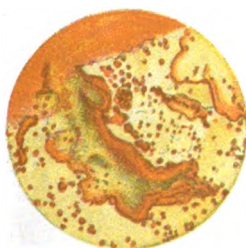


Fig 3

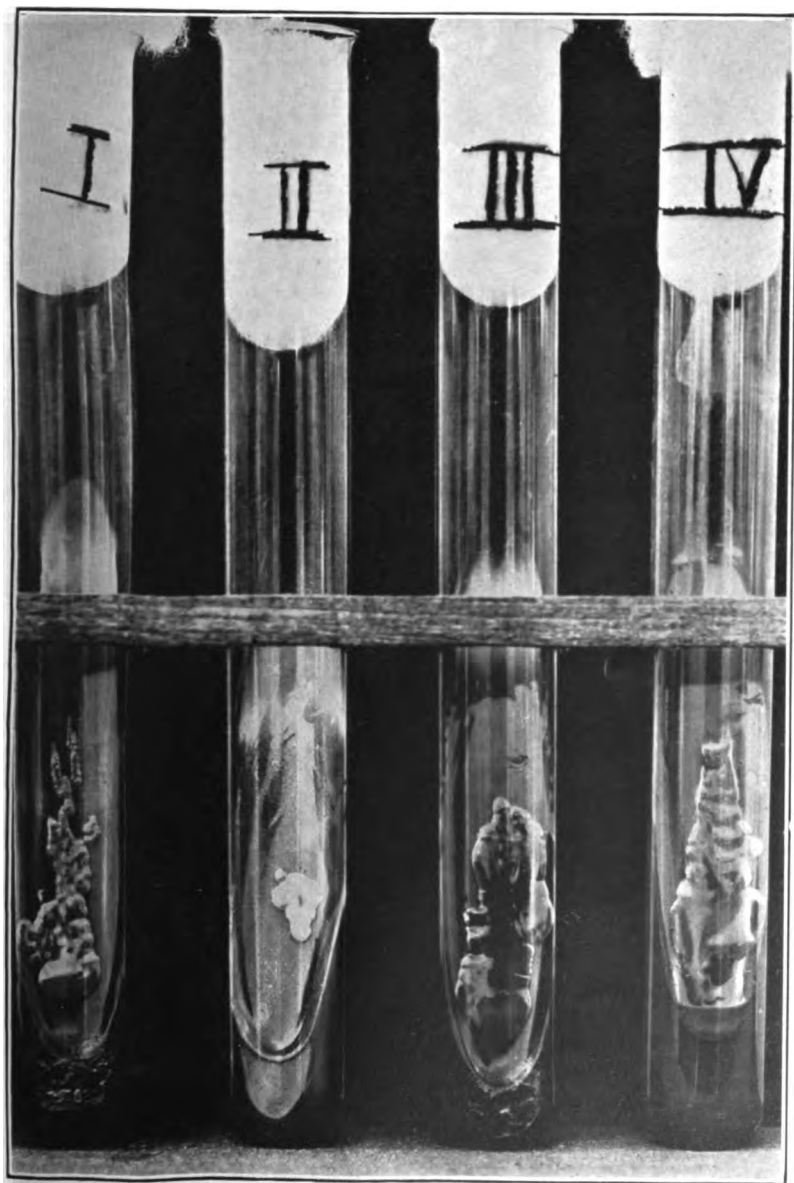


FIG. 4.—I, Glycerine-agar tube, twenty-four hours' growth; II, Lactose-agar tube, twenty-four hours' growth; III, Glycerine-agar tube, two days' growth, anaerobic; IV, Salt-agar tube, twenty-four hours' growth.

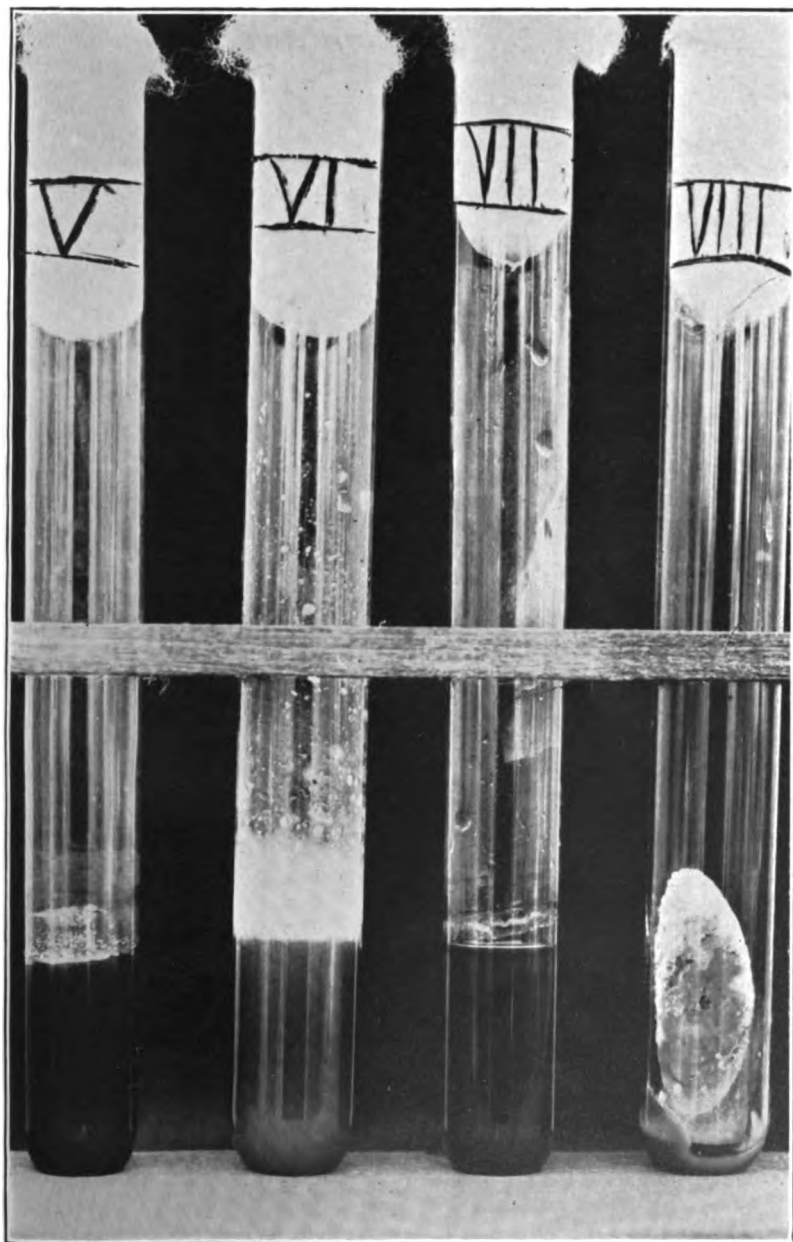


FIG. 5.—V, Litmus-lactose-agar tube, twenty-four hours' growth; VI, Litmus-milk tube, several days' growth; VII, Twenty per cent gelatine tube, twenty-four hours' growth; VIII, Potato tube, three days' growth.

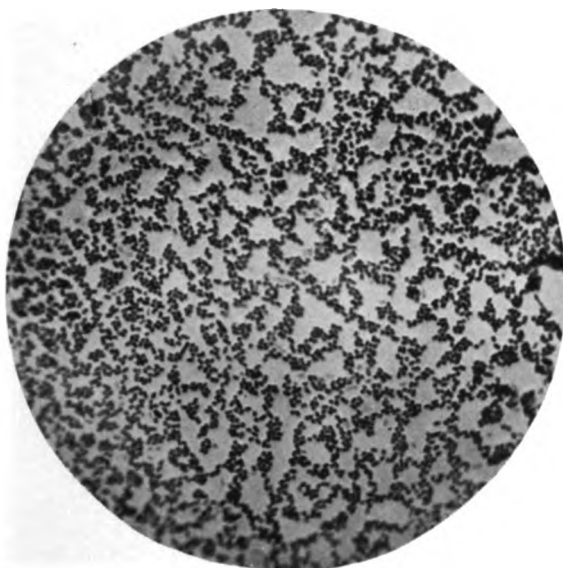


FIG. 6.—Microphotograph of a cover-glass preparation from an agar culture twenty-four hours old. Stained with dilute carbol-fuchsin. Zeiss Apochromatic homogenous oil immersion one-twelfth of an inch, compensation ocular No. 8. Length of bellows, 34 centimeters.

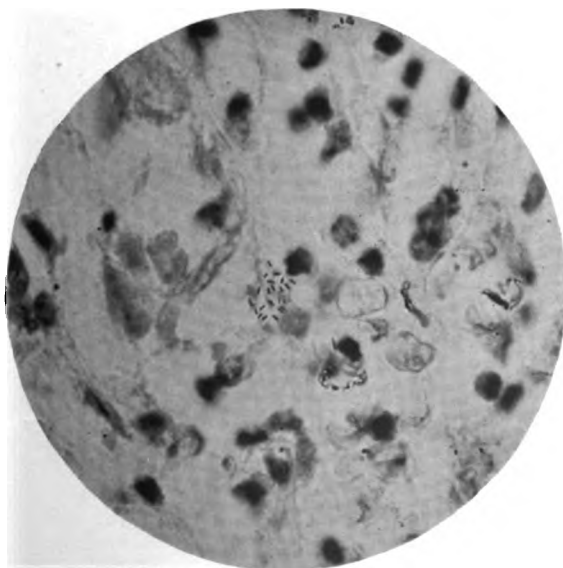


FIG. 7.—Microphotograph of an interstitial inflammatory focus in the kidney, showing a small mass of the bacilli. Magnification same as in fig. 6.

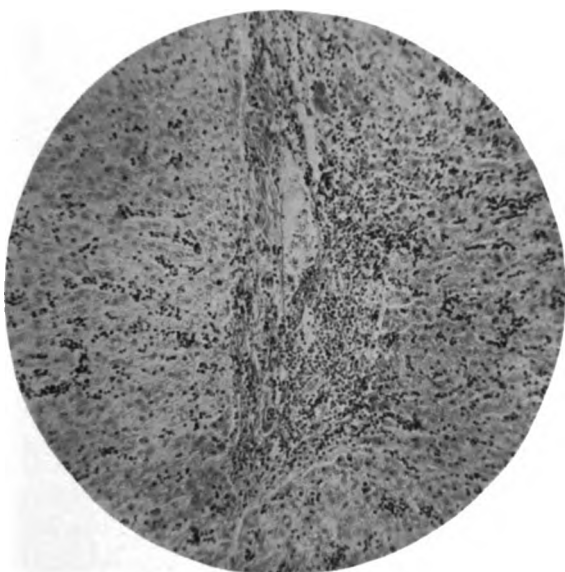


FIG. 8.—Microphotograph of an interlobular inflammatory periphlebitic focus in the liver. Zeiss objective A. A. compensation ocular No. 8.



FIG. 9.—Microphotograph of a degenerated glomerulus, almost complete fibrosis. Magnification same as in fig. 8.

A FATAL INFECTION BY A HITHERTO UNDESCRIBED CHROMOGENIC BACTERIUM: *BACILLUS AUREUS FÆTIDUS*.

By MAXIMILIAN HERZOG, M. D., *Pathologist, Biological Laboratory.*

The fauna and flora of the Tropics being in general different from those of the more temperate latitudes, in genera as well as in species, we may also reasonably expect this observation to hold good with reference to the very lowest forms of life—bacteria and protozoa—however, without being unmindful of the fact that certain families and even species are distributed over an enormous territory and under the most varied conditions of life.

We can hardly expect to find that bacteria and protozoa, which are strict parasites of widely distributed races, are limited in area, unless it be that they depend in certain stages of their life cycle upon an intermediate host, itself confined to certain regions. To cite an example: The tubercle bacillus is practically found wherever, like the human being, an easily susceptible host of this strict parasite dwells. The *Hæmamoeba malariae*, likewise a parasite of man, is not so widely distributed, because it depends for its spreading upon an intermediate host, the anopheles, the distribution of which is not identical with that of man.

The *a priori* deduction that in the Tropics we will find certain bacteria and protozoa peculiar to this zone will therefore be limited to those bacteria and protozoa which are either strictly saprophytic or parasitic in certain hosts, confined to the Tropics themselves, or which only occasionally and under particularly favorable circumstances lead a parasitic life.

The observation to be recorded in this paper refers to a case of fatal human infection by a hitherto undescribed bacterium, which is evidently not very pathogenic under ordinary conditions and probably, as a rule, is a harmless saprophyte, but which, as in this instance, under circumstances especially favorable, may become parasitic and may lead to a fatal issue. Examples of this type are of course not unknown in clinical medicine. The colon bacillus,

usually a harmless saprophyte, living in the intestinal contents, occasionally invades the juices and tissues of the human body and leads to acute or chronic fatal infections.

The case to be reported is as follows:

On the 19th of February, 1904, the body of D. L., a Filipino, 40 years of age, was sent to the morgue at San Lazaro. No more was known about the case than that the person died rather suddenly, the suspected cause of death being plague. There was an open wound on the right leg and a marked swelling of the inguinal glands of the right side. The post-mortem examination was made eight hours after death. Briefly the findings were as follows:

The body was that of a well-developed man of about 40 to 45 years of age. Post-mortem rigidity was well marked. Post-mortem lividity was extensive on dependent parts of the body and extending somewhat toward the sides of the neck and thorax. There was a swelling of the size of a hazelnut in the right inguinal region. The swollen area was firm and elastic. There was no fluctuation and no edema in the neighborhood. The skin was perfectly intact. There was no difference in color over this area and the surrounding tissues. In the right popliteal space there was an open ulcerated surface 5 by $1\frac{1}{2}$ centimeters in size, the long diameter being in the long axis of the limb. The ulceration was several millimeters deep, had very sharp, somewhat raised edges, and was covered with a small amount of sero-fibrinous exudate, which became visible after the removal of iodoform, which had been dusted freely on the wound. The open surface was covered with small granulations. The whole condition of the ulcer suggested that the surface had recently been curetted and its edges trimmed. The integument did not show any lesions other than the ulcer described.

On opening the body cavities it was found that the serous membranes were shining and transparent. There was a very small amount of the usual serous fluid in the thoracic and abdominal cavities. It may be stated here that no hemorrhagic spots, petechiae, or ecchymoses were found anywhere. The pericardium and heart appeared normal. The large vessels were also normal. The lungs were very slightly hyperemic, otherwise normal. Bronchi, trachea, and larynx were normal. Epiglottis was slightly injected. The spleen was normal in size, consistency, and color. The trabeculae were a little more marked than usual.

The kidneys showed a very marked injection. They were of a deep pinkish-purple hue. The capsules were smooth, even, and transparent and peeled off easily. After removal of the capsule the surface appeared finely granular, but the granular elevations were very faint. The glomeruli appeared strongly injected and were surrounded by a much paler, somewhat grayish-white tissue. On section the glomeruli stood out as deeply injected points, while the deeply injected vessels alternated with somewhat dull, grayish-white tubules. The pyramids were likewise of a deep pinkish-purple color. Relation between cortex and medulla was normal. The mucous membrane of the pelvis was smooth and shining. There was a very small amount of turbid urine in the pelvis. The suprarenals were normal.

The liver was normal in size and rather firm in consistency. The capsule on the whole was smooth and transparent. However, on the upper surface there were seen a few small, irregular, dull, slightly raised perihepatic areas. The surface was here and there slightly uneven and finely though very superficially granular. The color was a pinkish, grayish yellow. On section the color was somewhat deeper and brownish, but otherwise of the same hue as when seen through the capsule. The centers of the lobules were rather grayish white. The veins contained a large amount of dark purplish blood.

The gall bladder was normal and contained a moderate amount of turbid, greenish-yellow bile. The mucous membrane was smooth. There were no stones. The pancreas was perhaps somewhat firmer in consistency than usual, otherwise normal.

Stomach and small intestines: The mucosa was very slightly congested, otherwise normal. The large intestine was normal. The appendix was normal.

Lymph glands: Three of the inguinal glands of the right side were found to be markedly hypertrophic. They were not measured, but appeared to be enlarged to twice their normal size in all their diameters. On the left side they were very slightly enlarged. The markedly hypertrophic glands of the right side were quite firm in consistency, not injected, and rather pale. The cervical glands were slightly enlarged and very moderately congested.

Smears from the organs showed a small number of what appeared to be a small diplococcus or diplobacillus. No organisms showing the characteristic morphology and staining properties of the plague bacillus were found. It was therefore decided that the

case was not one of plague infection. This decision was reached shortly after the post-mortem examination, as it was necessary to determine at once the course to be taken in regard to the disposition of the body and the house from which it came.

The anatomical diagnosis of the post-mortem findings was as follows:

Passive congestion of the kidneys. Passive congestion of the liver. Acute interstitial hepatitis. Hypertrophy of the inguinal glands. Slight hypertrophy and slight congestion of the cervical glands.

Cause of death.—Remote; iodoform poisoning (?). Immediate; terminal diplococcus infection.

During the post-mortem examination glycerine agar tubes were inoculated with the usual precautions. The tubes were examined after two days with the following results :

Two tubes inoculated from the liver contained pure cultures of a short, small bacillus, which had produced a golden-yellow pigment.

One tube from the heart's blood developed the same organism, but it was, as shown on the third day, contaminated.

One tube inoculated from the spleen remained permanently sterile. None of the tubes developed the plague bacillus.

Before proceeding to a more detailed description of the organism isolated, it may here be stated that inquiries were made as to the history of the case and as to the possibility of iodoform poisoning. As is usual with the lower class of Filipinos, only a very fragmentary, unsatisfactory history could be obtained. Dr. Christensen, health inspector of the district from which the body was sent in for a post-mortem examination and diagnosis, had never seen the man alive, but learned that he had not been perfectly well for about four months, that he had an open wound on his leg, and that iodoform had been used freely on it for about eight days. No history of any symptoms of iodoform poisoning, such as mental depression, hallucinations, delirium, etc., could be obtained. While the bacteriologic and histologic examination of the case was in progress the liver was examined for iodine in the Chemical Laboratory, with negative results.

DESCRIPTION OF THE BACILLUS ISOLATED.

Morphology.—Short bacilli with rounded ends, varying much in size. They are from 0.6 to 2 microns long; on an average 1.4

microns. The larger individuals of two microns are rather scarce. In thickness the bacilli vary from 0.55 to 0.8 micron. They are generally about 0.7 micron thick. The proportion of the length to the thickness is usually 2:1. The organism presents itself frequently as a diplobacillus. A large number are short, making them look like diplococci. Occasionally small individuals are found, not materially larger than 0.5 micron and almost spherical, so that it is hard to distinguish them from true diplococci. The bacillus possesses a capsule of moderate size, which can be demonstrated by Muir's method. It does not form long chains, even groups of four in a chain being but rarely seen. Spore formation is not observed. It is not motile, but shows a lively molecular motion in hanging drop preparations.

Staining properties.—The bacillus is readily stained by the watery aniline stains and easily overstained by the more intense solutions (carbol-fuchsin, carbol-thionin). When properly treated not all of the bacilli take the stain uniformly, but in some cases the latter acts in such manner as to leave an unstained space in the center. It is not demonstrable that this polar staining is due to the presence of Ernst-Babes's polar granules, because Neiser's methylene-blue-Bismark-brown does not satisfactorily show any such granules, although there appears to be a slight tendency to take up some of the stain at the poles of the bacillus. When properly treated the organism somewhat, though rather remotely, resembles the short type of the pseudodiphtheria bacillus. A certain resemblance also exists between it and the bacillus of plague, though the similarity is not great. Gram's method decolorizes the bacillus. No flagellæ are demonstrable.

Cultural peculiarities.—The organism on all solid media which have been tried produces an intense golden-yellow pigment, which is practically identical in color with that formed by the *Staphylococcus pyogenes aureus*. Distinct colonies in 20 per cent gelatine plates are quite difficult to obtain, because the organism liquefies the media with great rapidity. Twenty per cent gelatine stab cultures after twenty-four hours to a great extent become fluid. The liquefaction comprises the entire extent of the upper strata. There is some growth in the depth along the line of the stab, but not much. The liquefied gelatine is very cloudy, and after twenty-four hours a dense scum is formed on its surface. Individual colonies are best studied on agar plates. On agar and glycerine

agar the organism forms a moist, raised, golden-yellow growth after twenty-four hours. The individual colonies are more or less round and likewise moist and raised, with a somewhat undulating surface. The margins are smooth. In spreading, the colonies become confluent. The development on glucose agar is identical with that on ordinary agar. No gas formation occurs. On 3 per cent salt agar the growth is possibly a little slower, although not very much so. The bacillus raised on this medium stains as usual; the involution forms so characteristic in the case of the plague bacillus are never seen. On lactose agar the growth is similar to that on plain agar. There is no gas formation. The development is more rapid on the surface than in the depth of the stab, but it also occurs there, although at a much slower rate. On lactose litmus agar the color begins to turn after twenty-four hours and is quite distinctly red after forty-eight hours. Bouillon after twenty-four hours is strongly clouded, and a scum has been formed on the surface after forty-eight hours. On potatoes a very typical luxuriant growth is observed after twenty-four hours. Litmus milk is slightly reddened after twenty-four hours and strongly so after forty-eight. Coagulation takes place only at the end of several days. The organism develops typically under anærobic conditions in either a nitrogen or a hydrogen atmosphere. All cultures whether ærobic or anærobic have a very fetid, cheesy, and somewhat cadaverous smell.

The thermal death point of the organism was determined to be 62° C. An exposure of ten minutes at this temperature destroyed all the bacilli, while 61° C. acting for ten minutes left a number alive.

The name "*Bacillus aureus fortidus*" selected for this micro-organism emphasizes two of its prominent characters, its chromogenic and malodorous properties.

No chromogenic bacterium described in Sternberg's Manual of Bacteriology, Flügge's *Die Mikroorganismen*, Migula's *System der Bakterien*, or the last volumes of the *Centralblatt für Bakteriologie* is identical with the bacillus described in this paper.

HISTO-PATHOLOGY.

Pieces of tissue taken at the post-mortem table were at once placed in Zenker's solution. They were subsequently embedded in paraffine and stained with hematoxylin-eosin, eosin-alkaline-

methylene-blue, and by Gram's method. The microscopic examination demonstrates the following tissue changes:

Liver.—The boundaries of the lobules are well marked, since the interlobular veins are surrounded by an inflammatory infiltration, the latter in general having the character of a periphlebitic cellular exudate. In a fair number of places this inflammatory process must have been going on for some time, since here the interlobular tissues show a number of fusiform connective tissue cells and fibers. When examined with oil-immersion magnification it is seen that the cellular exudate consists mainly of small round cells of the lymphoid type; here and there a plasma cell is seen. These plasma cells are of the ordinary type with a more or less square or irregular protoplasmic body, deeply staining with methylene blue and with an excentrically situated vesicular nucleus, poor in chromatin. The cellular exudate also shows fusiform cells of the type of fibroblasts, while a number of the small round cells show karyokinetic figures, demonstrating that a lively proliferation has been going on in the inflammatory foci. The latter exhibit a considerable number of very small bacilli found in irregular groups, in groups of two, and in small chains. This micro-organism does not generally stain very well, even with methylene blue. Some, however, keep the dye fairly well.

The liver cells show distinctly noticeable, though not very advanced, degrees of fatty degeneration. This degenerative process is perhaps most marked in the center of the lobules, though it is not confined to the central zone but may be quite diffusely distributed in some areas. The liver capillaries are distended with blood. The capsule of the liver in places shows some thickening. Where this condition prevails we find an interlobular inflammatory focus in the neighborhood of the capsule.

Kidneys.—The majority of the glomeruli appear normal; some, however, show an increase in the nuclei of the endothelial lining of the glomerular capillaries, while in others there is a more or less marked thickening of the capsules of Bowman. We also see beginning fibrosis in the interior of the tufts. In a number of the latter the fibrosis is well advanced, and we have a complete obliteration of the capillaries. In the neighborhood of the glomeruli, which show more or less advanced changes, and between the convoluted tubules there are seen inflammatory foci, which consist mostly of small round cells of the lymphoid type. However, there

are also present some plasma cells and plasma "mast cells," and a considerable number of eosinophilic polynuclears. These foci likewise show the small, generally poorly stained bacilli. The epithelial cells lining the uriniferous tubules show cloudy swelling or vacuolation with loss of the nucleus quite extensively. Most tubules contain a granular material; some contain hyaline casts. The renal blood vessels are all distended with blood.

Neither the liver nor the kidneys show any areas of marked or extensive coagulation necrosis nor are areas of blood extravasation encountered, though both the kidneys and the liver show highly engorged capillaries.

Lymph glands.—The inguinal lymph nodes show a marked increase in fibrous connective tissue. This increase is noticeable in the capsule, in the trabeculae and around the individual blood vessels. However, the follicles themselves show no marked fibroid changes, and the differentiation between the peripheral zone and the proliferating center of Fleming is well preserved. The lymphoid cells are of the usual character. Karyokinetic figures are here and there seen in the proliferating center of the follicles. The mitoses, however, are not very numerous. Occasionally an eosinophilic polynuclear is encountered. Plasma cells and plasma "mast cells" are also seen. Bacilli like those found in the liver and kidneys are encountered, nowhere in large groups or over large continuous areas, but only as a few isolated individuals here and there forming little groups among the cells. The blood vessels of the lymph nodes are generally well filled. Hemorrhagic areas of extravasated blood are not found.

No histologic changes are found in the pulmonary tissue. The pancreas is normal except in certain portions, where there is seen a minor degree of increase in the interlobular connective tissue.

The myocardium shows fragmentation of a marked degree, the diastases between the fragments not being very large.

A moderate number of cells exhibit a deposit of brown granules in the perinuclear zone. More or less all cells show a very fine, dust-like vacuolization and a somewhat indistinct, hazy striation.

ANIMAL EXPERIMENTS.

On February 26, 1904, at 9 a. m., a small monkey (*Macacus philippinensis*) was given an intraperitoneal injection of 2 to 3 c. c. of

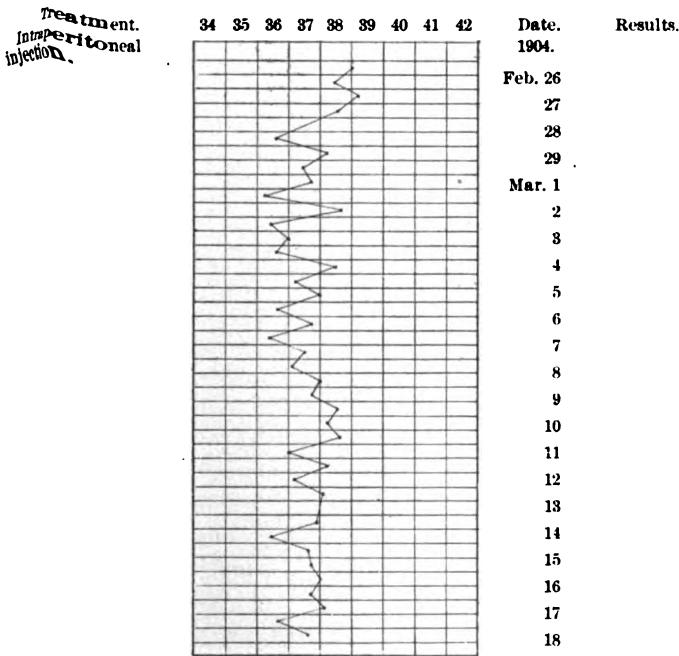
a twenty-four hours' bouillon culture of the bacillus. There was only a very slight reaction, as seen from the temperature chart, and the animal was well at the end of one month. (See Chart No. 1.)

On March 1, 1904, at noon, a good-sized, full-grown rabbit was given an intraperitoneal injection of 2 to 3 c. c. of an emulsion of a two days' agar culture in sterile water. The result was the same as in the case of the monkey. (See Chart No. 2.)

CHART No. 1.

Monkey No. 558, February 26, 1904.

[Weight, small; age, young; sex, female; inoculation, 2 to 3 c. c.; history, twenty-four hours' bouillon culture of bacillus aureus fetidus. Intraperitoneal.]



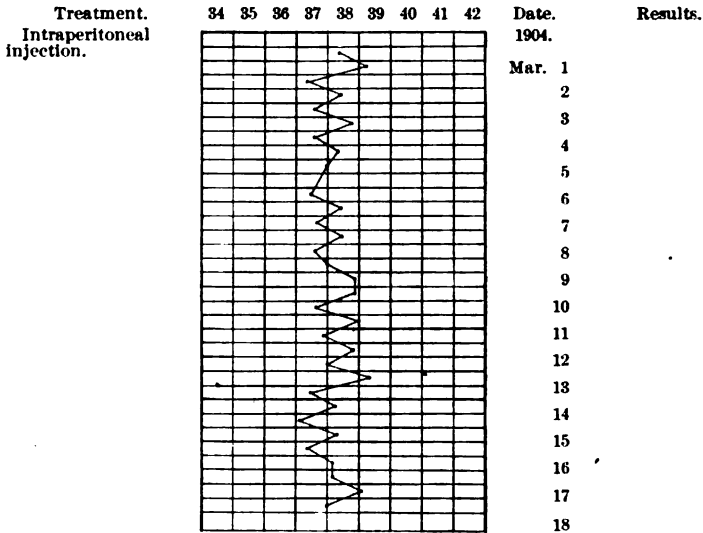
A similarly negative result was obtained with a half-grown wild gray rat inoculated subcutaneously with a platinum loop of a fresh agar growth.

As appears clearly from these animal experiments the bacillus aureus fetidus is not highly pathogenic, and in a single inoculation did not have a tendency to become parasitic.

CHART No. 2.

Rabbit No. 561, March 1, 1904.

[Age, adult; sex, female; color, white; inoculation, 2 to 3 c. c.; history, emulsion of bacillus aureus foetidus from two days' agar culture.]



CONCLUSIONS.

Bacillus aureus foetidus, the bacterium described in this paper, has, as appears beyond doubt, been the cause of death in the case herein reported. Experiments showed that the bacillus is not a highly pathogenic micro-organism, because single inoculations of moderate doses brought about only a very slight reaction in the animals experimented upon. Perhaps inoculations repeated during a longer period might bring about a more serious result.

It is very probable that *bacillus aureus foetidus* is ordinarily a saprophyte. In the case reported it may simply have lived for some time in the necrotic tissues of a neglected ulcer and may have slowly become modified in these environments until it finally gained entrance into the tissues of the patient. From the lymphatic system it entered the blood current, reached the liver and kidneys, and led to subacute and somewhat chronic interstitial fibroid processes and parenchymatous degeneration.

As shown by the microscopic examination, beginning interstitial and marked parenchymatous nephrites, as well as early, brown atrophy and fatty degeneration of the myocardium, must be added to the anatomical diagnosis made at the post-mortem table.



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1904.—No. 14.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

SERUM LABORATORY.

TEXAS FEVER IN THE PHILIPPINE ISLANDS AND THE FAR EAST.

By JAMES W. JOBLING, M. D., Director of the Serum Laboratory, and
PAUL G. WOOLLEY, M. D., Assistant Director Serum Laboratory.

BIOLOGICAL LABORATORY.
Bulletin No. 2 of the Entomological Division.

THE AUSTRALIAN TICK (*BOOPHILUS AUSTRALIS FULLER*) IN THE PHILIPPINE ISLANDS.

BY CHARLES S. BANKS, ENTOMOLOGIST.

MANILA:
BUREAU OF PUBLIC PRINTING.
1904.

17094

LETTERS OF TRANSMITTAL.

OFFICE OF THE SUPERINTENDENT
OF GOVERNMENT LABORATORIES,

Manila, April 14, 1904.

SIR: I have the honor herewith to transmit for publication as a bulletin of the Bureau two papers, one on "Texas Fever in the Philippine Islands and the Far East," by Jas. W. Jobling, M. D., and Paul G. Woolley, M. D., and the other on the "Australian Tick (*Boophilus australis* Fuller) in the Philippine Islands," by Charles S. Banks.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,
Secretary of the Interior.

BIOLOGICAL LABORATORY,

Manila, P. I., April 14, 1904.

SIR: I have the honor to transmit herewith and to recommend for publication a report on "The Australian Tick (*Boophilus australis* Fuller) in the Philippine Islands," by Mr. Charles S. Banks, Entomologist, Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

Dr. PAUL C. FREER,
Superintendent of Government Laboratories.

TEXAS FEVER IN THE PHILIPPINE ISLANDS AND THE FAR EAST.

By JAMES W. JOBLING, M. D., *Director of Serum Laboratory*, and PAUL G. WOOLLEY, M. D., *Assistant Director of Serum Laboratory*.

In the following report we shall not attempt to review the literature. The classic work of Smith and Kilbourne and the excellent reviews of Kossel, and Nocard and Laclainche render this unnecessary. We shall simply detail our own experiences.

Just before Texas fever appeared in Manila an attempt was being made to import American stock of medium size and good antecedents in order to improve the grade of native animals, which, through lack of care, had after many generations so degenerated that whether for slaughter, food, or dairy purposes, they were of little value.

In order not to introduce Texas fever into the Islands the cattle exported from the United States had been purchased at places 100 to 150 miles north of the Texas-fever line in California, all the animals having been raised at the places at which they were bought, with the exception of one heifer (No. 68), of which mention will be made. The herd was composed of two cows from San Bruno, a bull and three cows from Petaluma, a bull from Millbrae, and a bull and a heifer from San Jose.¹

On November 18, 1903, these cattle were landed in Manila. Prior to disembarkation they were examined and a few ticks were found, but these according to Veterinarian Meyers, were examples of a species other than that found in the Texas-fever belt of the United States. At this time, the animals appeared to be in good condition and not suffering from any acute or chronic disease.

Immediately upon landing they were sent to the Serum Laboratory for observation and for receiving anti-rinderpest treatment.

¹ The Laboratory numbers of the animals with relation to the place from which they came are as follows: Jersey bull No. 70 and Jersey heifer No. 68, from San Jose; Holstein bull No. 66, from Millbrae; Holstein cows Nos. 62 and 63, from San Bruno; Jersey cow No. 64, Ayrshire cows Nos. 65 and 69, and Ayrshire bull No. 67, from Petaluma.

At the time when "simultaneous inoculation" would have been practiced no virulent blood could be obtained and the cattle accordingly received prophylactic doses of immune serum only, so as to protect them until the necessary virulent material would be at hand.

The attached charts will show the temperature curves of these animals and will indicate their general condition better than words.

Twelve days after the second prophylactic dose of serum, virulent blood was obtained and "simultaneous inoculation" was given to all but one animal. The animal from which the virulent blood was taken was received from Shanghai on November 28. It developed rinderpest within a few days after arrival without having been inoculated, and was bled to death on December 3, 1903. The symptoms and post-mortem lesions were those always observed in rinderpest—that is, bloody diarrhea, redness of visible mucous membranes, and discharges from the eyes and nose. No pathologic change was found which could be attributed to Texas fever. The connective tissues were not yellow, the spleen was small and firm, the gall bladder enlarged and distended, and the mucous membrane of the fourth stomach inflamed and ulcerated. There were no symptoms which would lead us to think we had been using blood obtained from an animal suffering from Texas fever. This is the more certain since some of the blood was used not only to inoculate the American cattle but also eleven serum animals, the amounts varying from 1 to 1,000 c. c., and thirty calves received 5 to 50 c. c.

In all the cattle and calves other than the American cattle the reactions were those usually observed as following the inoculation of animals with virulent rinderpest blood. In no instance were there any symptoms which would lead us to think that we had been using blood from an animal suffering from Texas fever. This applies especially to some of the serum animals which received 1,000 c. c.

The results of this treatment in the cases of the American cattle were disastrous, for in a short time, varying in individual cases from four to eight days, the temperature rose abruptly to between 41° and 42° C., and five of the animals died.

The first one to succumb was No. 63. As this animal had developed a high temperature about the usual period following inoculation by the "simultaneous method," it was believed that death was caused by rinderpest, but the post-mortem showed none of the

lesions of this disease; instead, there was a marked yellowish discoloration of all the connective tissues and cloudy swelling of the liver, spleen, kidneys, and heart. The liver was the main organ affected. It showed chronic biliary cirrhosis following infection with liver flukes, suppurative and chronic obstructive cholangitis, and calciferous fluke cysts.

Nos. 63 and 69 died on the same day, but the disease was so unexpected that pyroplasmosis was not suspected until autopsy was made on No. 69, when the yellow color of the connective tissues and the size and consistence of the spleen, together with bloody urine, led Veterinarian Murray Meyers to make a diagnosis of Texas fever. The blood of the animal was not examined, but the following day another one (No. 66.) died, and in the blood corpuscles of the latter we were able to demonstrate the causative organisms in stained preparations.

Upon both of these animals and upon No. 70, ticks were found which were collected and sent to the entomologist of the Bureau who identified them as the Australian variety (*B. Australis*¹).

The clinical history taken from all the fatal cases was similar. No. 63 had been apparently well since landing and had been kept with the other cattle in a screened stable. Following the second injection of serum her temperature rose to 39.9° C., but fell during the next twenty-four hours to normal, where it remained until eight days later, when it again rose, this time to 40° C. This rise was but a transient one, and for the next week the curve was within normal limits. Twelve days after receiving the second dose of serum, the "simultaneous method" of immunization against rinderpest was practiced. On the fifth day following this the temperature was 40.5° C., on the sixth day it was 41.2° C., on the seventh day it was 41.6° C., and on the eighth day the animal died.

The autopsy showed all the connective tissues in a very yellow condition. The spleen was large and soft, and the mucous membrane of the gastro-intestinal tract in good condition. There were no ulcers in the abomasum. The liver was cloudy and honey-combed with abscesses containing flukes.

This history, save for some minor differences in the temperature

¹ See the paper on this subject by Mr. C. S. Banks.

curves and the fact that no other animals showed the same hepatic condition, applies very well to the other cattle dying of the disease—that is, Nos. 65, 66, 67, and 69.¹ (See charts.)

One animal (No. 62) had the usual rise of temperature, and up to the time of death no pyroplasmas could be demonstrated, but trypanosomas were present. Whether this one died of one or the other of the suggested diseases can not be surely stated. In animal No. 70 the usual rise of temperature occurred and the parasites appeared in the blood. Later the fever decreased and the animal made a quick and satisfactory recovery. Animal No. 68 at no time showed a high fever, but following the “simultaneous inoculation” the temperature remained consistently between 39° and 40° C., and subsequently fell and remained regularly between 38° and 39° C. Parasites were never found in this animal in spite of careful and painstaking daily examinations covering a period of about three weeks. In this case we can not say whether or not we were dealing with a subacute attack of pyroplasmosis, but we do know that this heifer was bought at San Jose with No. 70, but was not a native of that place, having been brought into the herd from some other district. In the case of No. 64, in which no virulent blood was used, there was no sign of Texas fever.

Besides these cases two others have occurred in Australian cattle which were said to come from a district where neither the ticks

¹A somewhat similar experience has been told us by Dr. H. E. Keylock, of Shanghai, who has generously permitted us to use his story.

Dr. Keylock was preparing antirinderpest serum at Shanghai, using Chinese cattle exclusively, and upon the appearance of rinderpest among some dairy cattle in his district had been requested to treat the sick and immunize the other animals of the dairy. In this establishment there were ten animals which had been raised for the purpose for which they had been used and had never been outside the grounds where they were kept. The sick were given 50 c. c. of serum on alternate days until they had received in all 150 c. c. The others were immunized by the “simultaneous method,” which in this case meant 50 c. c. of serum and 10 c. c. of virulent blood. Following this, just as in our cases in Manila, three of the animals developed hemoglobinuria and subsequently died. The time intervening between inoculation and the onset of symptoms we do not know. Not suspecting Texas fever, neither autopsies nor blood examinations were made. A portion of the same blood given in the cases of these dairy animals was used to inoculate other animals used for serum purposes, and with no bad results.

nor the disease itself had ever been seen. The cattle were temporarily immunized with serum, and were then at once shipped to Baguio, in Benguet Province. Some time after arriving in the mountains both died with symptoms of Texas fever and the organisms were found in stained specimens of the blood. Three other cases in Australian cattle, two of which were fatal, have been observed at the farm of Mr. Angel, just across the river from Santa Ana. All these animals showed the parasites, but in comparatively small numbers. The cases were subacute. Previous to the occurrence of these cases no similar ones had been observed, nor had the care takers noticed any ticks until three or four weeks before the outbreak.¹

The above facts immediately brought up the question as to the origin of the disease, which had never before been observed in the Philippine Islands, but which had developed in animals imported from north of the Texas-fever line in the United States, and then only after these animals had been inoculated with fresh blood from native or Chinese cattle.²

¹ For obtaining a brilliant differential stain for these parasites and the blood corpuscles, we have had the best success with the following method, which has the advantage of fixing and staining the smears in a very short time and the further advantage of not overstaining.

Dried blood stains are covered with a solution of Wright's methylene blue and eosin in methyl alcohol. After a minute enough water is added to form a scum on the surface of the liquid. This is allowed to remain in contact with the smear for from two to fifteen minutes and is then poured off. Loeffler's methylene blue solution is added and the preparation stained during one to five minutes. The slide is then washed with water and quickly rinsed with a one-fourth per cent solution of acetic acid, washed, dried, and mounted. It will be seen that this is simply Wright's modification of the Romanowsky method, with Theobald Smith's method appended. It gives very satisfactory results.

² In this connection the report of Lingard for 1902-3 is most interesting. He says that the disease was first recognized in India in 1871, when it was discovered clinically by Colonel Hallen. Lingard's own cases occurred after inoculation with virulent rinderpest blood, and agree with those of investigators in Africa, and he concludes that the animals which he used were harboring the parasites at the time of inoculation and that the disease only became active when the resistance of the animal was reduced by an attack of rinderpest following inoculation. Comparable deductions were drawn by Koch in South Africa. The case of the bull No. 2, reported below, seems to be explained in a similar way.

In order to answer this question it was necessary to discover whether the native or Chinese animals were immune to the disease and whether or not American cattle presumably not immune could be infected by injecting into them the blood from the healthy native or Chinese cattle. We say native or Chinese cattle because the bloods of the two races have become so inseparably mixed in the processes of past immunizations that if one race is resistant to the disease the other must be. We may include animals from Singapore, Borneo, Java, Cochin China, and Australia, the bloods of all of which have been used for immunizing purposes and for serum work, and which have also been treated with native and Chinese blood. While such inoculations have been promiscuous, we can trace all blood which we have used and can state exactly how it has been used and what results have been obtained with it. If Texas fever were present in some animals in these Islands, and if the majority of the native animals were not immune, then we should have produced and reproduced the disease. Yet in a long series of blood examinations which numbered thousands, and in very large number of autopsies, we have been unable to see anything which suggested bovine malaria.

In order to settle beyond doubt just what the conditions here were in regard to Texas fever we attempted to reproduce the disease in Chinese animals by injecting subcutaneously blood containing large quantities of pyroplasmas. For this experiment two animals were used, one a calf imported from Hongkong (No. 325), the other a cow (No. 290), imported from Shanghai. Both of these were negative. At the time of the inoculation the calf (No. 325) showed large numbers of ticks on its hide. (See charts Nos. 325 and 290.)

We next attempted to produce the disease by injecting blood taken from a healthy American animal, which had not been immunized by the "simultaneous method" and which showed no parasites, into a healthy Chinese one (No. 333). The result was absolutely negative.

Following this we took blood from healthy animals used for serum purposes and injected it subcutaneously into American animals which had not received the "simultaneous method." This series of experiments was positive in two cases, negative in one. In the negative case (bull No. 1) we suspected an acquired immunity and

later injected a large amount of parasite-containing blood, an experiment that was also negative and which apparently supported our supposition. Some weeks later one of the positive animals (bull No. 2) contracted rinderpest and died suddenly. This animal was also suffering from actinomycosis. At autopsy, in addition to the actinomycosis, lesions of both Texas fever and rinderpest were found, and the pyroplasmas were found in the blood, but in a very small percentage of the blood cells. It seems reasonable to suspect that in this case the acquired rinderpest was the cause of the flaring up of the latent Texas fever and that the combination of the two diseases killed the animal.

The facts adduced as a result of the experiences detailed above were, first, that native or Chinese animals could not be infected with Texas fever by subcutaneous inoculation with relatively large quantities of blood containing the living parasites; second, that susceptible, non-immune American animals would acquire the disease following injections of blood taken from apparently healthy Chinese animals which had been immunized to rinderpest in the Philippine Islands; third, that we are dealing with true Texas fever and not the atypical South African or Rhodesian fever; and fourth, that a tick (*Boophilus australis*), the intermediate host of the parasite of Australian pyroplasmosis, is present in these Islands.

Our conclusion, based upon these facts, is that Texas fever is endemic, not only in India but also in China, Java, Borneo, Cochin China, Singapore, and the Philippine Islands, and that the majority at least of all native and Chinese animals are immune to the disease.

SOME RECENT REVIEWS ON TEXAS FEVER.

Kossel, Kolle und Wasserman's Handbuch, 1903. I. 841.

Nocard et Lelainche—Maladies des Animaux. Paris, 1903.

Lingard, Annual Report Imperial Bacteriologist for 1902-3. Calcutta, 1903.

Schmidt, Arch. f. wiss. u. prakt. Tierhk., 1903. P. 65.

Koch, Journal Comp. Path. and Ther., 1903. 16, 273.

BUREAU OF GOVERNMENT LABORATORIES.

Record of Variations of Temperature beginning January 20, 1904, at the Serum Laboratory, Manila, P. I.

Animal No. 1

Taken from

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE					
January 20					F	American	Bureau of Agriculture					
Day of Month.	30	31	1	2	3	4	5	6	7	8	9	10
Time of Day.												

44

43

42

41

40

39

38

37

36

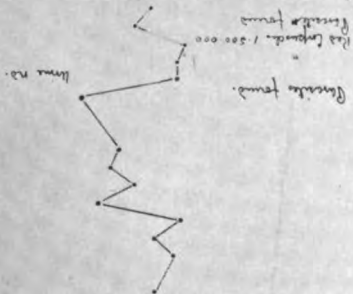
35

34

Inoculations.

Reaction.

Inoculations with 20 c.c. kept from Serum (Animal No. 251)

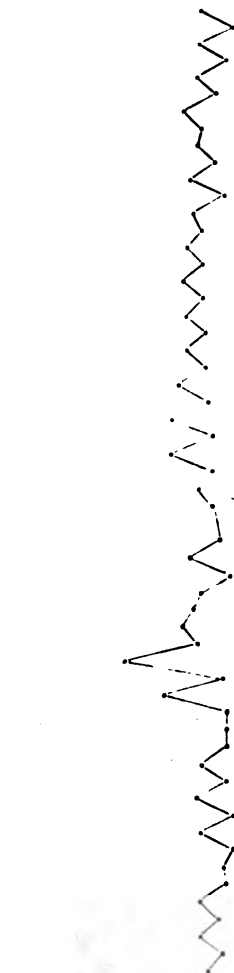


BUREAU OF GOVERNMENT LABORATORIES

Animal No. *Bull 7*

Record of Variations of Temperature beginning *January 4* *1904 at the Serum Laboratory, Manila, P. I.*

Day of Month	Kind of Animal	Height	Weight	Age	Color	Sex	Nativity	Reason of Acquisition	Prior
Jan. 4	Bull	10	11	12	13	14	15	16	17
5		18	19	20	21	22	23	24	25
6		26	27	28	29	30	31	32	33
7		34	35	36	37	38	39	40	41
8		42	43	44	45	46	47	48	49
9		50	51	52	53	54	55	56	57
10		58	59	60	61	62	63	64	65
11		66	67	68	69	70	71	72	73
12		74	75	76	77	78	79	80	81
13		82	83	84	85	86	87	88	89
14		90	91	92	93	94	95	96	97
15		98	99	100	101	102	103	104	105
16		106	107	108	109	110	111	112	113
17		114	115	116	117	118	119	120	121
18		122	123	124	125	126	127	128	129
19		130	131	132	133	134	135	136	137
20		138	139	140	141	142	143	144	145
21		146	147	148	149	150	151	152	153
22		154	155	156	157	158	159	160	161
23		162	163	164	165	166	167	168	169
24		170	171	172	173	174	175	176	177
25		178	179	180	181	182	183	184	185
26		186	187	188	189	190	191	192	193
27		194	195	196	197	198	199	200	201
28		202	203	204	205	206	207	208	209
29		210	211	212	213	214	215	216	217
30		218	219	220	221	222	223	224	225
31		226	227	228	229	230	231	232	233
1		234	235	236	237	238	239	240	241
2		242	243	244	245	246	247	248	249
3		250	251	252	253	254	255	256	257
4		258	259	260	261	262	263	264	265
5		266	267	268	269	270	271	272	273
6		274	275	276	277	278	279	280	281
7		282	283	284	285	286	287	288	289
8		290	291	292	293	294	295	296	297
9		298	299	300	301	302	303	304	305
10		306	307	308	309	310	311	312	313
11		314	315	316	317	318	319	320	321
12		322	323	324	325	326	327	328	329
13		330	331	332	333	334	335	336	337
14		338	339	340	341	342	343	344	345
15		346	347	348	349	350	351	352	353
16		354	355	356	357	358	359	360	361
17		362	363	364	365	366	367	368	369
18		370	371	372	373	374	375	376	377
19		378	379	380	381	382	383	384	385
20		386	387	388	389	390	391	392	393
21		394	395	396	397	398	399	400	401
22		402	403	404	405	406	407	408	409
23		410	411	412	413	414	415	416	417
24		418	419	420	421	422	423	424	425
25		426	427	428	429	430	431	432	433
26		434	435	436	437	438	439	440	441
27		442	443	444	445	446	447	448	449
28		450	451	452	453	454	455	456	457
29		458	459	460	461	462	463	464	465
30		466	467	468	469	470	471	472	473
31		474	475	476	477	478	479	480	481
1		482	483	484	485	486	487	488	489
2		490	491	492	493	494	495	496	497
3		498	499	500	501	502	503	504	505
4		506	507	508	509	510	511	512	513
5		514	515	516	517	518	519	520	521
6		522	523	524	525	526	527	528	529
7		530	531	532	533	534	535	536	537
8		538	539	540	541	542	543	544	545
9		546	547	548	549	550	551	552	553
10		554	555	556	557	558	559	560	561
11		562	563	564	565	566	567	568	569
12		570	571	572	573	574	575	576	577
13		578	579	580	581	582	583	584	585
14		586	587	588	589	590	591	592	593
15		594	595	596	597	598	599	600	601
16		602	603	604	605	606	607	608	609
17		610	611	612	613	614	615	616	617
18		618	619	620	621	622	623	624	625
19		626	627	628	629	630	631	632	633
20		634	635	636	637	638	639	640	641
21		642	643	644	645	646	647	648	649
22		650	651	652	653	654	655	656	657
23		658	659	660	661	662	663	664	665
24		666	667	668	669	670	671	672	673
25		674	675	676	677	678	679	680	681
26		682	683	684	685	686	687	688	689
27		690	691	692	693	694	695	696	697
28		698	699	700	701	702	703	704	705
29		706	707	708	709	710	711	712	713
30		714	715	716	717	718	719	720	721
31		722	723	724	725	726	727	728	729
1		730	731	732	733	734	735	736	737
2		738	739	740	741	742	743	744	745
3		746	747	748	749	750	751	752	753
4		754	755	756	757	758	759	760	761
5		762	763	764	765	766	767	768	769
6		770	771	772	773	774	775	776	777
7		778	779	780	781	782	783	784	785
8		786	787	788	789	790	791	792	793
9		794	795	796	797	798	799	800	801
10		802	803	804	805	806	807	808	809
11		810	811	812	813	814	815	816	817
12		818	819	820	821	822	823	824	825
13		826	827	828	829	830	831	832	833
14		834	835	836	837	838	839	840	841
15		842	843	844	845	846	847	848	849
16		850	851	852	853	854	855	856	857
17		858	859	860	861	862	863	864	865
18		866	867	868	869	870	871	872	873
19		874	875	876	877	878	879	880	881
20		882	883	884	885	886	887	888	889
21		890	891	892	893	894	895	896	897
22		898	899	900	901	902	903	904	905
23		906	907	908	909	910	911	912	913
24		914	915	916	917	918	919	920	921
25		922	923	924	925	926	927	928	929
26		930	931	932	933	934	935	936	937
27		938	939	940	941	942	943	944	945
28		946	947	948	949	950	951	952	953
29		954	955	956	957	958	959	960	961
30		962	963	964	965	966	967	968	969
31		970	971	972	973	974	975	976	977
1		978	979	980	981	982	983	984	985
2		986	987	988	989	990	991	992	993
3		994	995	996	997	998	999	1000	1001



Temperature with 20 c.c. blood for
7 hours with 20 c.c. blood for
7 hours with 20 c.c. blood for

34
Temperature
Reaction

BUREAU OF GOVERNMENT LABORATORIES.

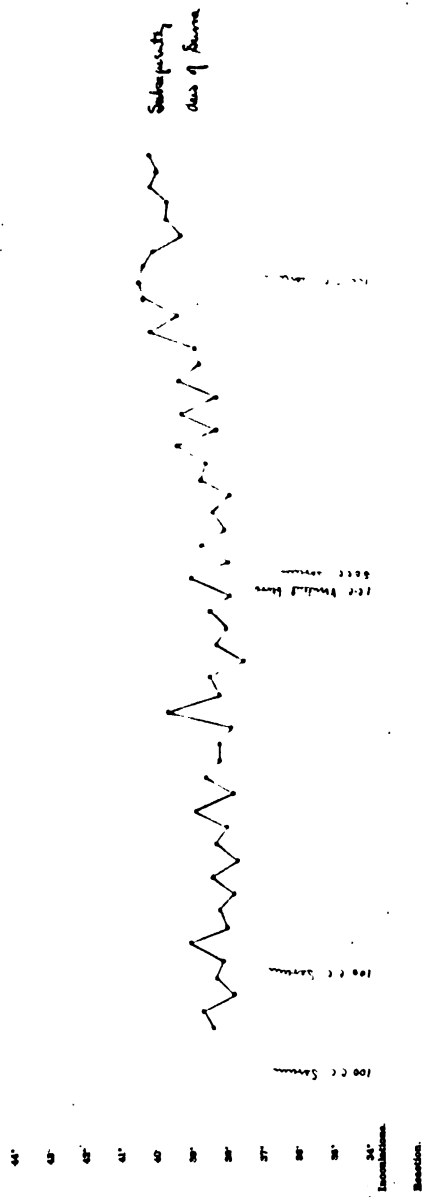
Animal No 64

Time 10:00

Record of Variations of Temperature beginning November 14, 1903, at the Serum Laboratory, Manila, P. I.

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE
Cow	50	1000	4 yrs	Black & white	F	Mountain	San Bruno Cal

Day of Month
November
Time of Day



100 C. Serum

100 C. Serum

100 C. Serum

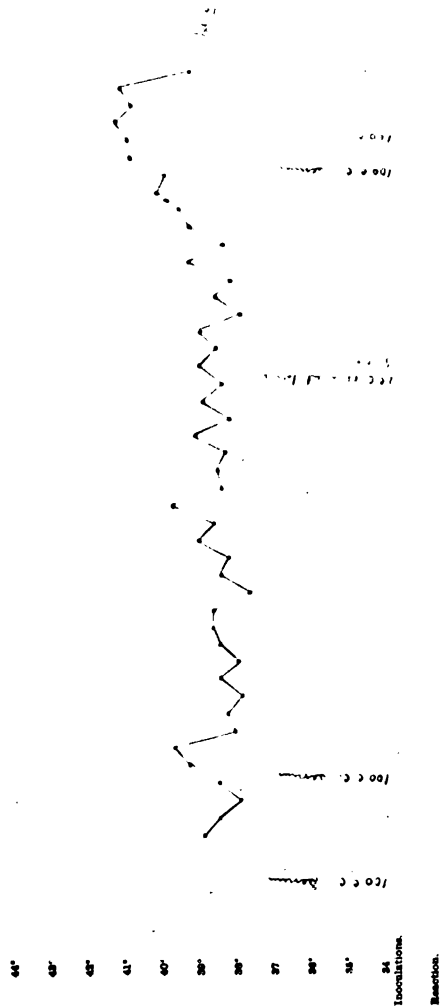
100 C. Serum

Temperature
Reaction

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Animal No. 5

Record of Variations of Temperature beginning

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BUREAU OF GOVERNMENT LABORATORIES.

Animal No. 64

1903, at the Serum Laboratory, Manila, P. I.

November

Record of Variations of Temperature beginning

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE
Cow	4'8"	700	6 yrs	Brown	F	Manila, Cal.	

Day of Month.

Temperature

Time of Day.

44°

43°

42°

41°

40°

39°

38°

37°

36°

35°

34°

Localizations.

Reaction



10:00 C

10:00 C

10:00 C

10:00 C

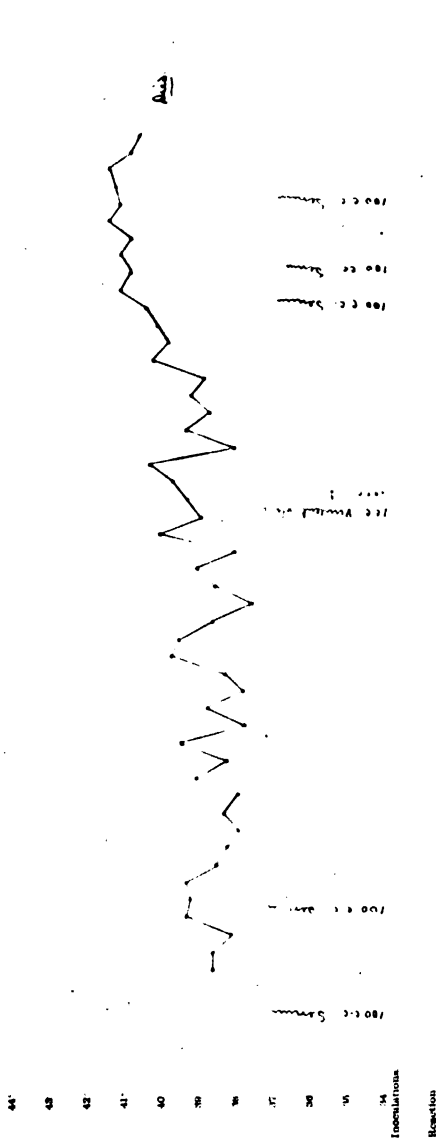
BUREAU OF GOVERNMENT LABORATORIES.

Animal No 65

Record of Variations of Temperature beginning November 10, 1903, at the Serum Laboratory, Manila, P. I.

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	ACTIVITY	FEED
Cow	4 1/2	100	6 yrs	Red and White	♀	Understand	Philippines ... Cal.

Day of Month.
Temperature
Time of Day.



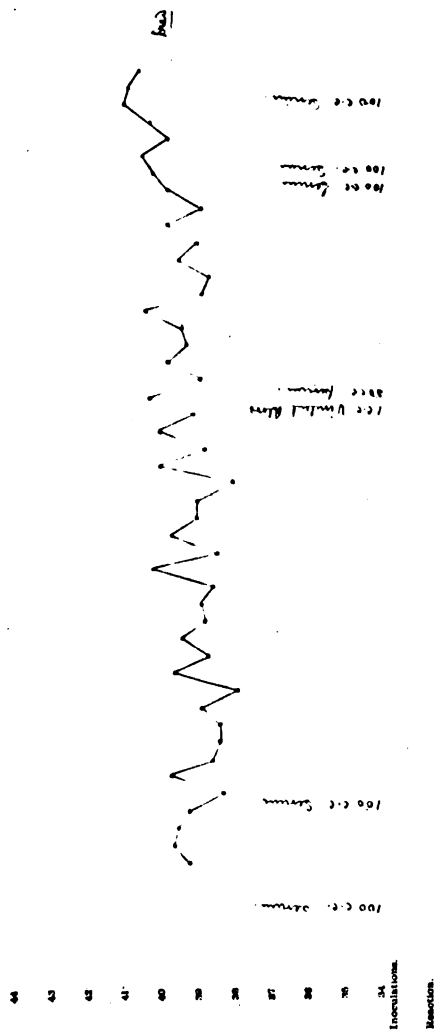
Animal. Vol. 66

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Record of Variations of Temperature beginning

November 13.

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE
pure	43"	100	3 yrs	Black with	M	German	Medium size



BUREAU OF GOVERNMENT LABORATORIES.

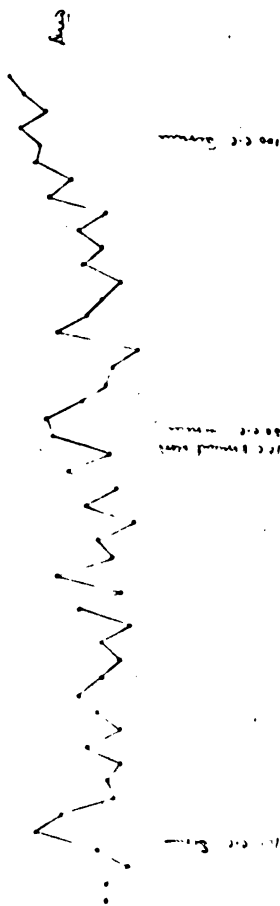
Animal No. 67

Record of Variations of Temperature beginning December 19, 1903, at the Serum Laboratory, Manila, P. I.

KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE
11	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
12	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
13	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
14	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
15	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
16	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
17	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
18	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
19	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
20	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
21	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
22	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
23	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
24	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
25	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
26	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
27	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
28	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
29	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
30	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
31	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
32	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
33	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
34	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
35	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
36	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
37	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
38	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
39	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
40	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
41	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
42	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
43	44"	7.00	3 yrs	Red	Male	American	Philippine Co.
44	44"	7.00	3 yrs	Red	Male	American	Philippine Co.

Day of Month

Time of Day



Time of Day

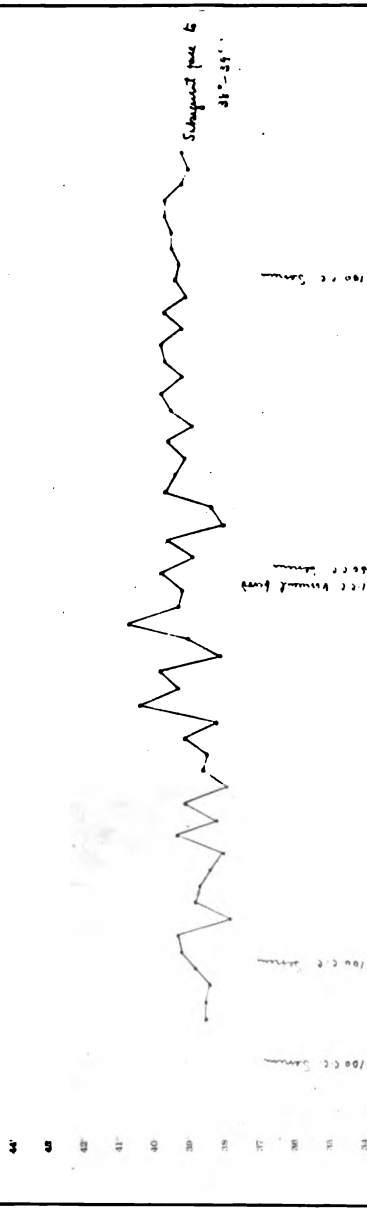
Time of Day

BUREAU OF GOVERNMENT LABORATORIES.

Two

**Record of Variations of Temperature beginning November 19 1903, at the Serum Laboratory, Manila, P. I.*

NO.	KIND OF ANIMAL.	HEIGHT.	WEIGHT.	AGE.	COLOR.	SEX.	NATIVITY.	PRICE.
18	19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34	35
36	37	38	39	40	41	42	43	44
45	46	47	48	49	50	51	52	53
54	55	56	57	58	59	60	61	62
63	64	65	66	67	68	69	70	71
72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89
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513	514	515	516	517	518	519	520	521
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639	640	641	642	643	644	645	646	647
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747	748	749	750	751	752	753	754	755
756	757	758	759	760	761	762	763	764
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792	793	794	795	796	797	798	799	800
801	802	803	804	805	806	807	808	809
810	811	812	813	814	815	816	817	818
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855	856	857	858	859	860	861	862	863
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873	874	875	876	877	878	879	880	881
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909	910	911	912	913	914	915	916	917
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936	937	938	939	940	941	942	943	944
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963	964	965	966	967	968	969	970	971
972	973	974	975	976	977	978	979	980
981	982	983	984	985	986	987	988	989
990	991	992	993	994	995	996	997	998
999	1000	1001	1002	1003	1004	1005	1006	1007
1008	1009	1010	1011	1012	1013	1014	1015	1016
1017	1018	1019	1020	1021	1022	1023	1024	1025
1026	1027	1028	1029	1030	1031	1032	1033	1034
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1044	1045	1046	1047	1048	1049	1050	1051	1052
1053	1054	1055	1056	1057	1058	1059	1060	1061
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1098	1099	1100	1101	1102	1103	1104	1105	1106
1107	1108	1109	1110	1111	1112	1113	1114	1115
1116	1117	1118	1119	1120	1121	1122	1123	1124
1125	1126	1127	1128	1129	1130	1131	1132	1133
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1152	1153	1154	1155	1156	1157	1158	1159	1160
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1179	1180	1181	1182	1183	1184	1185	1186	1187
1188	1189	1190	1191	1192	1193	1194	1195	1196
1197	1198	1199	1200	1201	1202	1203	1204	1205
1206	1207	1208	1209	1210	1211	1212	1213	1214
1215	1216	1217	1218	1219	1220	1221	1222	1223
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1233	1234	1235	1236	1237	1238	1239	1240	1241
1242	1243	1244	1245	1246	1247	1248	1249	1250
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1287	1288	1289	1290	1291	1292	1293	1294	1295
1296	1297	1298	1299	1300	1301	1302	1303	1304
1305	1306	1307	1308	1309	1310	1311	1312	1313
1314	1315	1316	1317	1318	1319	1320	1321	1322
1323	1324	1325	1326	1327	1328	1329	1330	1331
1332	1333	1334	1335	1336	1337	1338	1339	1340
1341	1342	1343	1344	1345	1346	1347	1348	1349
1350	1351	1352	1353	1354	1355	1356	1357	1358
1359	1360	1361	1362	1363	1364	1365	1366	1367
1368	1369	1370	1371	1372	1373	1374	1375	1376
1377	1378	1379	1380	1381	1382	1383	1384	1385
1386	1387	1388	1389	1390	1391	1392	1393	1394
1395	1396	1397	1398	1399	1400	1401	1402	1403
1404	1405	1406	1407	1408	1409	1410	1411	1412
1413	1414	1415	1416	1417	1418	1419	1420	1421
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1431	1432	1433	1434					

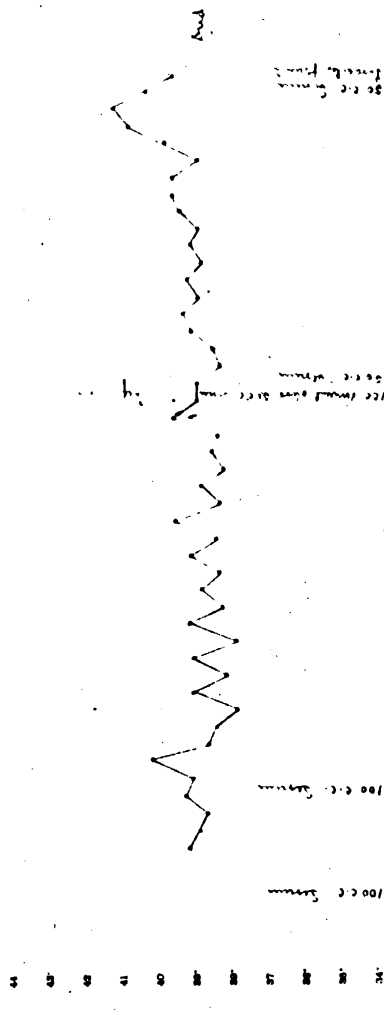
[illegible]

BUREAU OF GOVERNMENT LABORATORIES.

Animal No. 29

Record of Variations of Temperature beginning 190... at the Serum Laboratory, Manila, P. I.

	KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	PRICE							
	Cow	4-6	700	2 yrs	Black										
Day of Month	1	14	16	1	2	3	4	5	6	7	8	9	10	11	12
Time of Day															

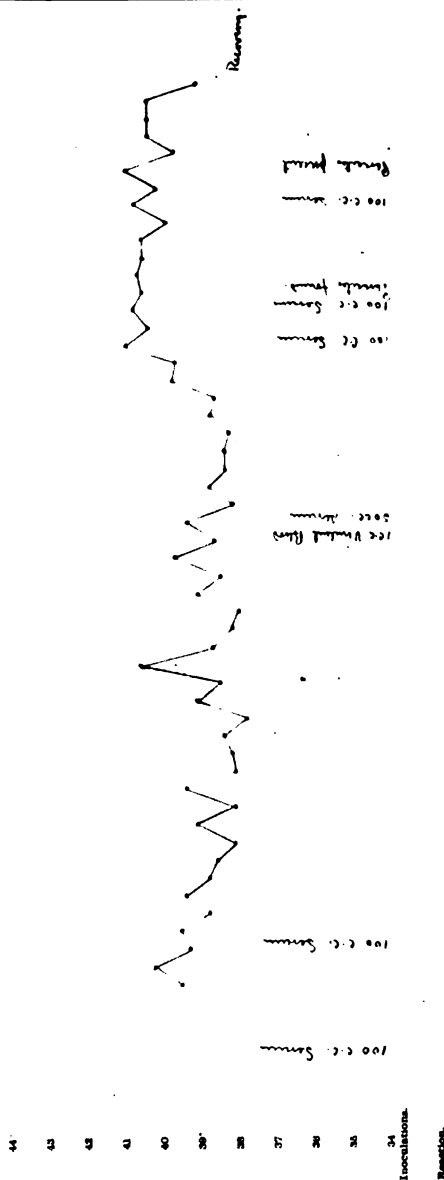


BUREAU OF GOVERNMENT LABORATORIES.

Animal No 70

Record of Variations of Temperature beginning November 19, 1903, at the Serum Laboratory, Manila, P. I.

DATE	KIND OF ANIMAL	HEIGHT	WEIGHT	AGE	COLOR	SEX	NATIVITY	FEED																						
	Bull	48"	500	4 yrs	brn	M	American	Cell																						
Day of Month.	15	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Temperature																														
Time of Day.																														



BUREAU OF GOVERNMENT LABORATORIES.

Animal No. 490

Town Time

Record of Variations of Temperature beginning *December 15*, 1903, at the Serum Laboratory, Manila, P. I.

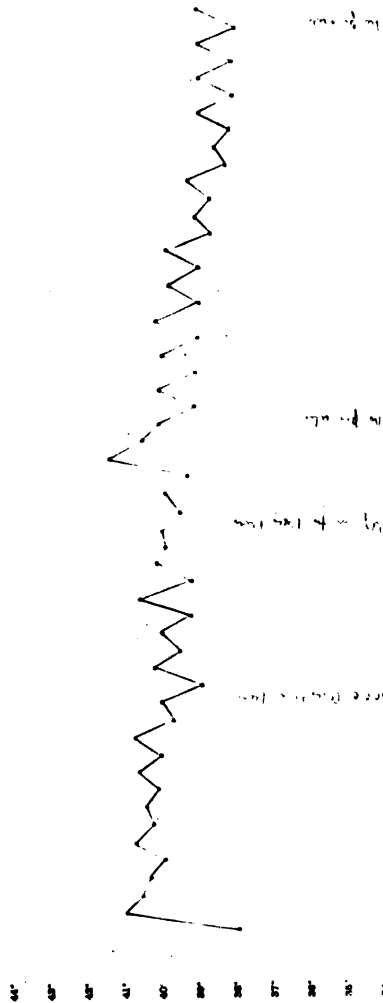
KIND OF ANIMAL.	HEIGHT.	WEIGHT.	AGE.	COLOR.	SEX.	NATIVITY.	PRICE.
Cow	46	800	4 yrs	Red	+	Single	
15	16	17	18	19	20	21	22
23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38
39	40	41	42	43	44	45	46
47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62
63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78
79	80	81	82	83	84	85	86
87	88	89	90	91	92	93	94
95	96	97	98	99	100	101	102
103	104	105	106	107	108	109	110
111	112	113	114	115	116	117	118
119	120	121	122	123	124	125	126
127	128	129	130	131	132	133	134
135	136	137	138	139	140	141	142
143	144	145	146	147	148	149	150
151	152	153	154	155	156	157	158
159	160	161	162	163	164	165	166
167	168	169	170	171	172	173	174
175	176	177	178	179	180	181	182
183	184	185	186	187	188	189	190
191	192	193	194	195	196	197	198
199	200	201	202	203	204	205	206
207	208	209	210	211	212	213	214
215	216	217	218	219	220	221	222
223	224	225	226	227	228	229	230
231	232	233	234	235	236	237	238
239	240	241	242	243	244	245	246
247	248	249	250	251	252	253	254
255	256	257	258	259	260	261	262
263	264	265	266	267	268	269	270
271	272	273	274	275	276	277	278
279	280	281	282	283	284	285	286
287	288	289	290	291	292	293	294
295	296	297	298	299	300	301	302
303	304	305	306	307	308	309	310
311	312	313	314	315	316	317	318
319	320	321	322	323	324	325	326
327	328	329	330	331	332	333	334
335	336	337	338	339	340	341	342
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415	416	417	418	419	420	421	422
423	424	425	426	427	428	429	430
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447	448	449	450	451	452	453	454
455	456	457	458	459	460	461	462
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535	536	537	538	539	540	541	542
543	544	545	546	547	548	549	550
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623	624	625	626	627	628	629	630
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687	688	689	690	691	692	693	694
695	696	697	698	699	700	701	702
703	704	705	706	707	708	709	710
711	712	713	714	715	716	717	718
719	720	721	722	723	724	725	726
727	728	729	730	731	732	733	734
735	736	737	738	739	740	741	742
743	744	745	746	747	748	749	750
751	752	753	754	755	756	757	758
759	760	761	762	763	764	765	766
767	768	769	770	771	772	773	774
775	776	777	778	779	780	781	782
783	784	785	786	787	788	789	790
791	792	793	794	795	796	797	798
799	800	801	802	803	804	805	806
807	808	809	810	811	812	813	814
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823	824	825	826	827	828	829	830
831	832	833	834	835	836	837	838
839	840	841	842	843	844	845	846
847	848	849	850	851	852	853	854
855	856	857	858	859	860	861	862
863	864	865	866	867	868	869	870
871	872	873	874	875	876	877	878
879	880	881	882	883	884	885	886
887	888	889	890	891	892	893	894
895	896	897	898	899	900	901	902
903	904	905	906	907	908	909	910
911	912	913	914	915	916	917	918
919	920	921	922	923	924	925	926
927	928	929	930	931	932	933	934
935	936	937	938	939	940	941	942
943	944	945	946	947	948	949	950
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959	960	961	962	963	964	965	966
967	968	969	970	971	972	973	974
975	976	977	978	979	980	981	982
983	984	985	986	987	988	989	990
991	992	993	994	995	996	997	998
999	1000	1001	1002	1003	1004	1005	1006
1007	1008	1009	1010	1011	1012	1013	1014
1015	1016	1017	1018	1019	1020	1021	1022
1023	1024	1025	1026	1027	1028	1029	1030
1031	1032	1033	1034	1035	1036	1037	1038
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1047	1048	1049	1050	1051	1052	1053	1054
1055	1056	1057	1058	1059	1060	1061	1062
1063	1064	1065	1066	1067	1068	1069	1070
1071	1072	1073	1074	1075	1076	1077	1078
1079	1080	1081	1082	1083	1084	1085	1086
1087	1088	1089	1090	1091	1092	1093	1094
1095	1096	1097	1098	1099	1100	1101	1102
1103	1104	1105	1106	1107	1108	1109	1110
1111	1112	1113	1114	1115	1116	1117	1118
1119	1120	1121	1122	1123	1124	1125	1126
1127	1128	1129	1130	1131	1132	1133	1134
1135	1136	1137	1138	1139	1140	1141	1142
1143	1144	1145	1146	1147	1148	1149	1150
1151	1152	1153	1154	1155	1156	1157	1158
1159	1160	1161	1162	1163	1164	1165	1166
1167	1168	1169	1170	1171	1172	1173	1174
1175	1176	1177	1178	1179	1180	1181	1182
1183	1184	1185	1186	1187	1188	1189	1190
1191	1192	1193	1194	1195	1196	1197	1198
1199	1200	1201	1202	1203	1204	1205	1206
1207	1208	1209	1210	1211	1212	1213	1214
1215	1216	1217	1218	1219	1220	1221	1222
1223	1224	1225	1226	1227	1228	1229	1230
1231	1232	1233	1234	1235	1236	1237	1238
1239	1240	1241	1242	1243	1244	1245	1246
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1263	1264	1265	1266	1267	1268	1269	1270
1271	1272	1273	1274	1275	1276	1277	1278
1279	1280	1281	1282	1283	1284	1285	1286
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1295	1296	1297	1298	1299	1300	1301	1302
1303	1304	1305	1306	1307	1308	1309	1310
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1327	1328	1329	1330	1331	1332	1333	1334
1335	1336	1337	1338	1339	1340	1341	1342
1343	1344	1345	1346	1347	1348	1349	1350
1351	1352	1353	1354	1355	1356	1357	1358

BUREAU OF GOVERNMENT LABORATORIES.

Animal No 333

Record of Variations of Temperature beginning December 15, 1903, at the Serum Laboratory, Manila, P. I.

Day of Month.	Kind of Animal.	Weight.	Height.	Age.	Color.	Sex.	Activity.	Price.
4	10	11	12	13	14	15	16	17
5	10	11	12	13	14	15	16	17
6	10	11	12	13	14	15	16	17
7	10	11	12	13	14	15	16	17
8	10	11	12	13	14	15	16	17
9	10	11	12	13	14	15	16	17
10	10	11	12	13	14	15	16	17
11	10	11	12	13	14	15	16	17
12	10	11	12	13	14	15	16	17
13	10	11	12	13	14	15	16	17
14	10	11	12	13	14	15	16	17
15	10	11	12	13	14	15	16	17
16	10	11	12	13	14	15	16	17
17	10	11	12	13	14	15	16	17
18	10	11	12	13	14	15	16	17
19	10	11	12	13	14	15	16	17
20	10	11	12	13	14	15	16	17
21	10	11	12	13	14	15	16	17
22	10	11	12	13	14	15	16	17
23	10	11	12	13	14	15	16	17
24	10	11	12	13	14	15	16	17
25	10	11	12	13	14	15	16	17
26	10	11	12	13	14	15	16	17
27	10	11	12	13	14	15	16	17
28	10	11	12	13	14	15	16	17
29	10	11	12	13	14	15	16	17
30	10	11	12	13	14	15	16	17
31	10	11	12	13	14	15	16	17



24
Examinations.
Remarks.

THE AUSTRALIAN TICK (*BOOPHILUS AUSTRALIS* FULLER) IN THE PHILIPPINE ISLANDS.

By CHARLES S. BANKS, *Entomologist Biological Laboratory.*

On the 18th of November, 1903, a consignment of cattle from the United States was landed in Manila. About three weeks afterwards they exhibited symptoms which were diagnosed as those of Texas fever, and in a few days several of the animals died of this disease. Conclusive proofs of the identity of the malady with Texas fever were established at the post-mortem examinations of the first animals which succumbed. I was thereupon directed to look for, and, if possible, to identify, any ticks which might be upon the remaining ones. Several ticks were picked from the animals on shipboard at the time of their arrival in Manila by a veterinarian who, from a preliminary examination, took them to be *Boophilus annulatus*, but who, after a microscopical one, modified his original statement. He did not, however, retain the specimens, so that I had no chance to see them and to identify them positively. I first saw the cattle after they had been in Manila for twenty-six days. Since landing they had been kept in fly-proof stables at the Serum Laboratory, being taken out only once or twice to be washed. The ticks which I secured may not have been of the same kind as those brought over by the animals from the United States and which were picked off by the veterinarian. These cattle may have carried with them *Boophilus annulatus*, but the latter may all have been removed while on shipboard, as the herd was carefully scrutinized before being landed. The ones which I found and identified as *Boophilus australis* Fuller may possibly have been picked up by the animals while they were being driven into the yards and stables. Ticks have a wonderful sticking power. This is due to the admirable adaptation of their feet to

the purpose of clinging to their hosts. By reference to figs. 11 and 16 the structure of the feet may be seen.

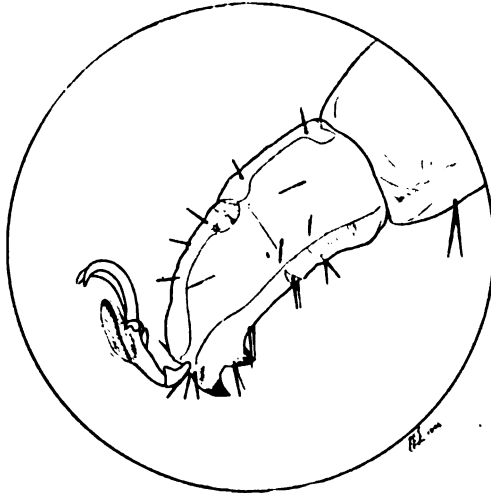


Fig. 16

Boophilus australis Fuller: Distal segment of tarsus of male, showing claws and pulvillum greatly enlarged. (Original.)

LIFE HISTORY AND DESCRIPTION.

Cattle ticks, one of the most annoying and serious of the parasites affecting domestic animals, because of their proven rôle in the transmission of disease, especially of Texas fever, belong to the family *Ixodidae*. This family of the order *Arachnida* is characterized as follows: They are hexapod in the larval and octopod in the nymph and adult stages. (See figs. 17 and 18, which represent the larvæ, and figs. 1 to 10, which show the adults.) In both the male (figs. 3-6) and the female (figs. 1, 2, 8-10) it is very difficult to distinguish the head from the rest of the body. That part of the female which appears as a brown, chitinous shield (figs. 9, 10, 14) at the fore part of the body is called the "capitulum" and corresponds to the head of true insects. It consists of a dorsal shield (fig. 9) terminating anteriorly in the rostrum (fig. 9b) or mouth parts which consist of the mandibles (fig. 10a), the maxillæ, and the palpi (fig. 10b). The maxillæ do not show in the drawing.

The palpi, as seen at *b* in fig. 10 and also in figs. 5*a*, 18, 19, and 20, are short, thick, and four-jointed, the segments being broader than they are long and having the folds thickened with chitin (see fig. 20, which represents the palpi of the young). They thus have a decidedly angular appearance. The mouth proper consists of a corrugated sheath (fig. 20*a*), in which the two lance-like, barbed, or hooked mandibles (figs. 12 and 13) move antero-posteriorly. This sheath bears upon its dorsal surface a series of very regular minute denticles and upon the ventral surface eight rows of the same of a larger size and pointing posteriorly (fig. 21). This number of ventral denticles is constant in *Boophilus annulatus* and *Boophilus australis*, but may be smaller or greater in other species. In the larval stage of *B. australis* the number is four (see fig. 20). The purpose of these denticles, judging from their structure and position, is evidently to give the animal a hold in the skin of its host. The mandibles are two bilaterally arranged, lancet-like organs, composed of chitin and having a barbed or hooked extremity (fig. 12*c*), with a moveable toothed appendage on the outer edge (fig. 12*e*). They also have a slight toothed growth near their point (fig. 12*d*).

The tick, when once fastened upon its host, does not relax its hold until replete with blood. This is true of the female. From observation of the male I am of the opinion that it does not attach itself in any one position for a great period, but wanders around upon the body of the host in search of the females. Its claws are so well adapted to clinging that it is removed only with considerable difficulty from the hairs among which it runs. Frequently the males are found adhering to the skin of the cattle.

The life history of cattle ticks is very interesting. The fecund female, after becoming engorged with blood, as shown in fig. 8, drops from the host and within twenty-four hours begins laying her eggs. These are tiny, brown, globular bodies, about, 0.75 millimeters in diameter, a very small white patch developing upon one side just previous to hatching. In the Laboratory, eggs which were laid on December 22, 1903, hatched January 25, 1904; but this period was no doubt longer than normal, owing to the unfavorable conditions under which the eggs were placed. They were laid in a Petri dish which was not supplied with the requisite amount of moisture.

After the young ticks had hatched, they remained clustered together among the eggshells for three or four days, after which they began to disperse. One morning, four or five days after hatching, I found several of them crawling over my table, and upon examination of the dish in which they had been placed I found that they had escaped in large numbers from beneath the supposedly tight lid. (See figs. 17 and 18, which show the young ticks very much enlarged.)

Under natural conditions the females deposit their eggs, in numbers estimated to be from 1,000 to 2,000, in some sheltered spot. Ticks exposed to strong sunlight do not hatch. Within a few days or a week after the young emerge from the eggs they climb to some favorable position on weeds or fences, and from there are easily brushed off upon the passing cattle. During this time they molt twice, changing from the hexapod to the octopod stage. These insects have frequently been met with in Manila upon posts, weeds, and the walls of houses and upon stone walls in and around places where cattle are kept. From the readiness with which they grasp any passing object, it is very probable that attendants might easily carry them upon their clothing into even a supposedly insect-proof stable.

Upon attaching themselves to an animal, if they have reached the adult stage, they immediately begin sucking the blood. Copulation also at once commences, and it is not an uncommon thing to find a male and a female *in copula* upon the same spot on the animal's skin, both of the ticks having their beaks or rostra inserted into it.

In this latitude, under normal conditions, the time elapsing from the time of laying the eggs to the maturing of the ticks is probably not more than seven weeks. The exact time can not be stated because of lack of sufficient observation.

HISTORY AND CLASSIFICATION.

The Australian tick is also known as the South American, the Cuban, or the Porto Rican Texas-fever tick, it having been thoroughly established that this animal as well as the American form, to which it is so very closely related, is capable of transmitting Texas fever.

Salmon and Stiles in their excellent work on "Cattle Ticks," in

the Seventeenth Report of the Bureau of Animal Industry of the United States Department of Agriculture for 1900 (1902) say:

The Australian and South American fever ticks were originally considered as identical with *B. annulatus*. When the Australian Commission visited this Bureau the writers of the present paper examined the Australian forms and expressed the opinion that they were certainly a distinct variety and in all probability a distinct species. The same specimens were examined by Ashmead, Schwarz, and Coquille, who concurred in this view. This opinion was referred to by our Australian colleagues in one of their publications. (Hunt and Collins, 1896, pp. 31, 32.) Neumann, who examined the same specimens upon which we based our view, looked upon the Australian form as identical with the North American. Fuller (1899, pp. 389-394), however, restudied the question and recognized the North American, the Australian, and the South African forms as three distinct species. The following extracts from this paper bear upon the question involved:

"Together with this I am sending you some notes on the various cattle ticks, from which you will see that I have found the North American, the Australian, and that from Cape Colony distinct from one another. The Queensland form appears to be a new species, for which I have proposed the name *australis*; it is curious that it is the same as the one Mr. Pond sent me as coming from South America.

* * * * *

"As early as 1893 the Queensland cattle tick was identified as *Ixodes boris* Riley, by the late A. S. Olliff, and was until recently regarded as specifically identical with that species by many later students. I believe that the first doubt as to the correctness of this assumption was thrown out by Dr. D. E. Salmon, Chief of the U. S. A. Bureau of Animal Industry, in a letter to Mr. P. R. Gordon, chief inspector of stock (Queensland). In this communication (dated December 9, 1897) Dr. Salmon says: 'You will possibly recall that we considered the Australian form as distinct from our American form. Professor Neumann, who had for his monograph a very large number of specimens, including our entire collection, and has studied the Australian ticks which Dr. Hunt gave us some time ago, does not, however, agree with us on this point, but considers that they are identical.

"As it has since become important to settle the identity of the supposed red-water tick in Cape Colony, also said to be *I. boris*, I have made a careful study of all three forms and have come to the conclusion that they are three distinct species.'"

The technical description of *B. australis*, as made by Mr. Fuller in the report of Salmon and Stiles, is here given. He, however, placed this tick in the genus *Rhipicephalus*, proposed by Neumann

in 1897. But as the species *Boophilus* differs from *Rhipicephalus* in the essentials of form, palpal joints, and stigmata, together with other structural features, recent authors have rather agreed to cling to the name given by Curtice in 1891 and which "has become almost vernacular."

THE AUSTRALIAN, SOUTH AMERICAN, CUBAN, AND PORTO RICAN TEXAS-FEVER TICK.

Boophilus australis Fuller.

SPECIFIC DIAGNOSIS.

Boophilus, male.—Body oval, narrowed in front, broadest (about 1.3 mm.) in region of stigmata and Coxæ IV, 2.2 to 2.3 mm. long. Scutum reddish brown, extends from anterior to posterior margin of body, but leaves a narrow lateral margin uncovered, prolonged in front by two pairs of projections; one pair of more prominent dorso-lateral pointed projections, dorsal of anterior projections of Coxæ I, and one pair less prominent and more median, somewhat semilunar, with concavity median, surrounding the base of the neck. Two shallow cervical furrows, extending more or less distinctly to the posterior margin of the body, may be interrupted in the middle; a median furrow present in posterior half, may be indistinct; festoons of posterior margin very indistinct. Distinct circular pores with extruding short, stout, bristly hairs scattered over the entire surface. Eyes small, pale at I intercoxal space. Ventral surface lighter than dorsal, all portions provided with short, stout hairs. Genital pore broad, transverse between Coxæ II. Anus slightly posterior of stigmal plane; and plates (clypei) more chitinous than those of *B. annulatus*. Strong chitinous cervical median caudal appendage present, 85 microns long. Capitulum about 425 microns long, postero-lateral spines of base more marked than in *B. annulatus*; mandibles about 600 microns, digit about 90 microns; internal apophysis apparently with three teeth; external apophysis bidentate (only three specimens examined). Hypostome with four rows of teeth on each half, the inner row being less strongly developed than the others. Palpi about 200 microns long, in general similar to those of *annulatus*. Legs strong; Coxæ similar to those of *annulatus*; but bidentation of I pair much more marked. Tarsi similar to *R. annulatus*.

Female.—Very similar to female of *B. annulatus*, but the lateral constriction at the stigma is usually more marked. Body elliptical, as broad in front as in back; when replete, may attain 10 to 12 mm. long by 6 to 7 mm. broad. Color varies like *annulatus*. Dorsal shield smaller, somewhat lighter in color. Eyes small, near middle or anterior third of lateral margin of scutum. Capitulum very short, 560 to 612 microns from postero-dorsal margin to anterior end of hypostome, quite similar to that of *annulatus*, except lateral projection slightly more marked. Mandibles 765 microns long, digit 120 microns; internal apophysis "tricuspid," and

"presenting rounded process as well;" external apophysis tridentate as in *annulatus*. Hypostome spatulate, slightly longer than palpi, each half with four rows of 7 to 10 denticles which do not extend to the base. Palpi very short, about 320 microns long, articles at least as broad as long, in general similar to *annulatus*.

Hexapod larva: Similar to *R. annulatus*; in some cases a third pair of stigmata (between Coxæ I and II) appears to be present.

Rhipicephalus australis, species nova.

Female.—When replete, measuring 10 to 11 mm. in length and 6 to 7 mm. in breadth. Dorsal shield smaller than that of *annulatus* and greater than that of *decoloratus*, of the same form and with similar furrows. Eyes, pale. Labium with eight rows of teeth.¹ Mandibles with lesser process tricuspid, and presenting a rounded process as well.

Male, adult.—Approaching that of *annulatus*, but with adanal shields more chitinous and also exhibiting a caudal appendage. Neither the shields nor the "tail" are so pronounced as those of *decoloratus*.

Habitat.—On horses, cattle, etc.; northwest to northeast Australia.



Fig. 21.

Diagram of labium of *Boophilus australis* and *B. annulatus*. Greatly enlarged. (Redrawn from Fuller.)

In addition to the habitat above given we may now add the Philippine Islands, inasmuch as the ticks found upon cattle here have been identified as belonging to this species.

It is well to direct the attention of those who are likely to be called upon to make a diagnostic examination of the Australian tick to the essential points of difference between *B. annulatus* and *B. australis*.

Aside from the fact that *B. australis* has been found in the Philippine Islands and that *B. annulatus* has not, the differen-

¹ See fig. 21.

tiation of the two species by those who study specimens may be facilitated by the following table, modified from Fuller's report:

- | | |
|---|----------------------|
| A. Mandibles with lesser process bicuspid..... | <i>B. annulatus.</i> |
| B. Mandibles with lesser process tricuspid, or quadricuspid,
or having a fourth slight rounded projection..... | <i>B. australis.</i> |
| | |
| A. Male with anal (adanal) chitinous plates slightly toothed
on the median posterior margin ¹ | <i>B. australis.</i> |
| B. Male with anal (adanal) chitinous plates more evenly
rounded ¹ | <i>B. annulatus.</i> |
| | |
| A. Male with a distinct horny tail..... | <i>B. australis.</i> |
| B. Male with no evidence of a tail..... | <i>B. annulatus.</i> |
| | |
| A. Larvæ having a curved line posterior to eyes and inclosing
dorsally a semilunar space comprised by the posterior
part of the body..... | <i>B. australis.</i> |
| B. Larvæ having no curved division line..... | <i>B. annulatus.</i> |

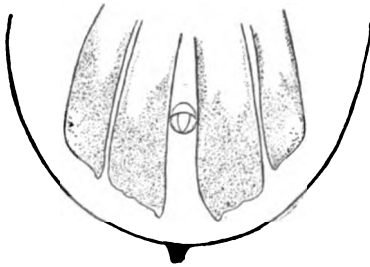


Fig. 22.

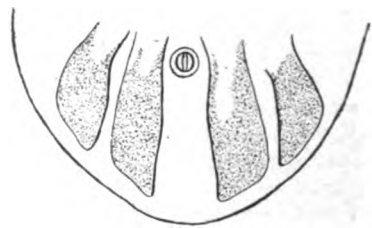


Fig. 23.

Diagrams of posterior margins of males of *Boophilus australis* and *B. annulatus*, showing caudal appendages in former and toothed appearance of adanal plates. Greatly enlarged. (Fig. 22 redrawn from nature, fig. 23 redrawn from Salmon and Stiles.)

In the female there is unfortunately no mark of distinction for the two species, the surest evidence in this case being the finding of males, as above described, in copula with females on the same host.

¹ See figs. 22 and 23, which give a diagrammatic representation of the adanal plates of *B. australis* and *B. annulatus*.

THE AUSTRALIAN CATTLE TICKS.

Boophilus australis, Fuller.

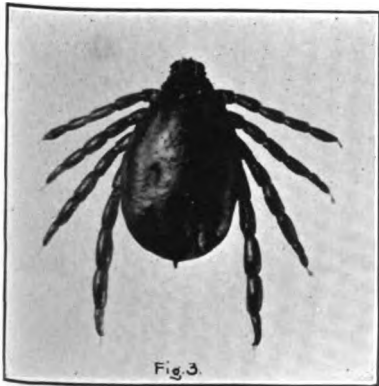
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1899. *Rhipicephalus australis* Fuller (1899), pp. 389-394, figs. 1-3. Notes on the Queensland Cattle Tick and Its Relationship to the Texas Fever Tick and the Blue Tick of Cape Colony (South Africa). Queensland Agric. Jour., Brisbane, IV (pt. 5), May.
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1901. *Boophilus australis* (Fuller), Salmon, D. E., and Ch. W. Stiles, 1901, pp. 426-433, figs. 114-151, 153d, 154c. The Cattle Ticks of the United States (*Ixodida*), Seventeenth Annual Report of the Bureau of Animal Industry, U. S. Dept. Agric., Washington, pp. 380-488, figs. 42-357.

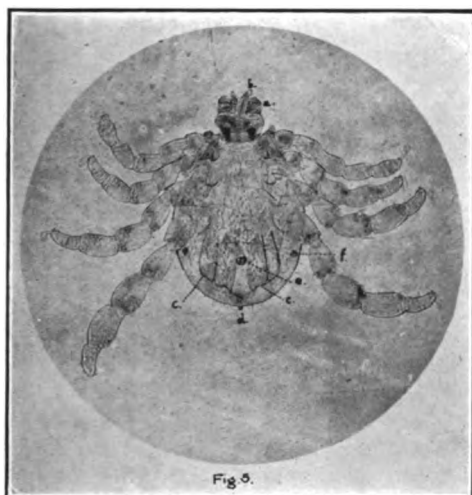
¹ This date reference can not be found. It is evidently intended to be: Canestrini, G., and F. Fanzago, 1877. Intorno agli acari Italiani. Atti del reale Instituto Veneto descienze, lettere ed arti, ser. 5, IV (1877-1878) [for 1877], pp. 69-208, Tav. II-VII.



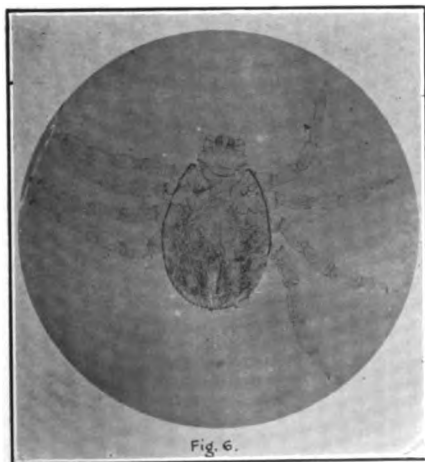
Boophilus australis Fuller: Adult female, dorsal and ventral aspects; specimens not yet replete. (Fig. 1, 8 X; fig. 2 slightly more.)



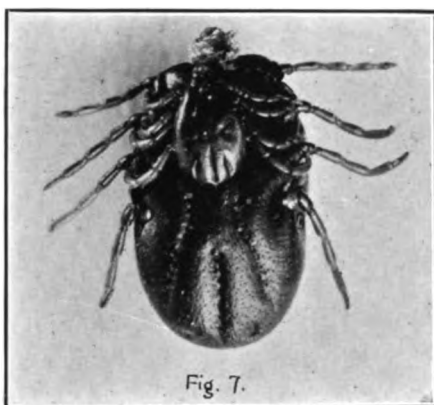
Boophilus australis Fuller: Adult male, dorsal and ventral aspects, showing caudal appendage or "tail." (13½ X.)



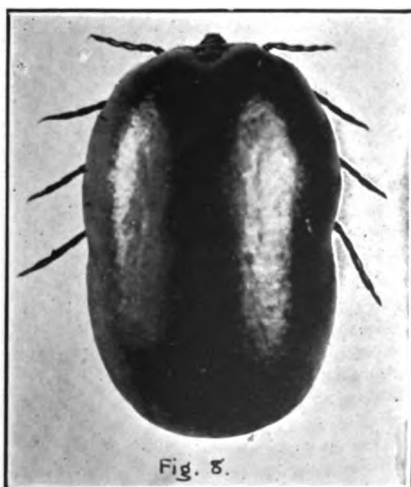
Boophilus australis Fuller: Adult male, ventral aspect. (9 X.) a, Palpi; b, Mandibles; c, Adanal plates; d, Caudal appendage, "tail;" e, Anus; f, Stigma.



B. australis Fuller: Adult male, dorsal aspect, showing hairs and minute denticles of proboscis. (9 X.)



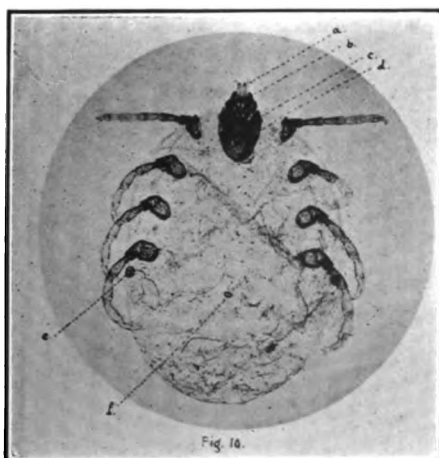
B. australis Fuller: Adult male and female in copula. (8 X.)



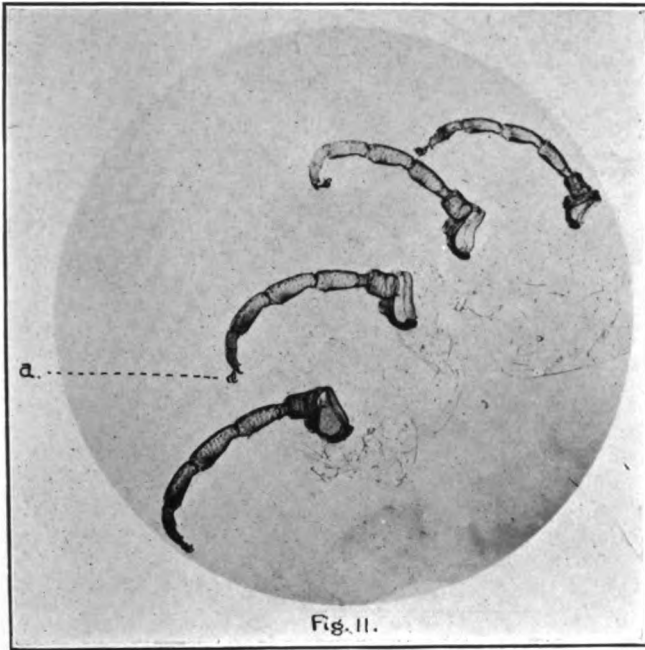
B. australis Fuller: Replete adult female, showing lateral constriction. (5½ X.)



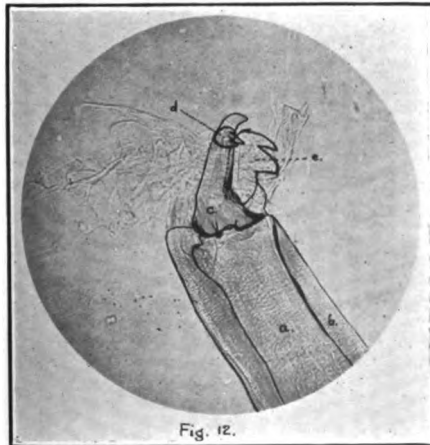
Boophilus australis Fuller: Half-grown female. (15 X.) a, Scutellum; b, Rostrum; c, Stigmata.



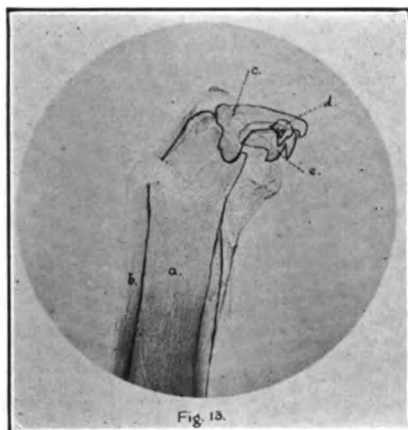
B. australis Fuller: Adult female previous to repletion. (6 X.) a, Mandibles; b, Palpi; c, Scutellum; d, Ocelli (eyes); e, Stigma; f, Anus.



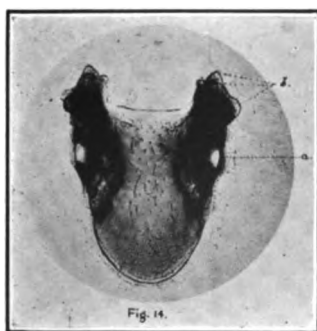
Boophilus australis Fuller: Left legs of female, showing claws. (12 X.)



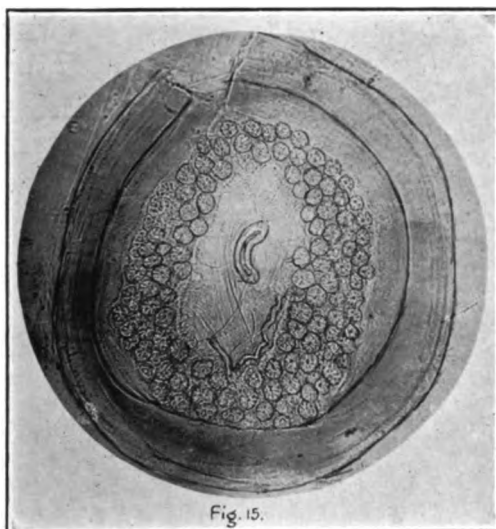
Boophilus australis Fuller: Mandible in sheath. (100 X.) a, Mandible; b, Sheath; c, Digit of mandible; d, Internal apophysis; e, External apophysis.



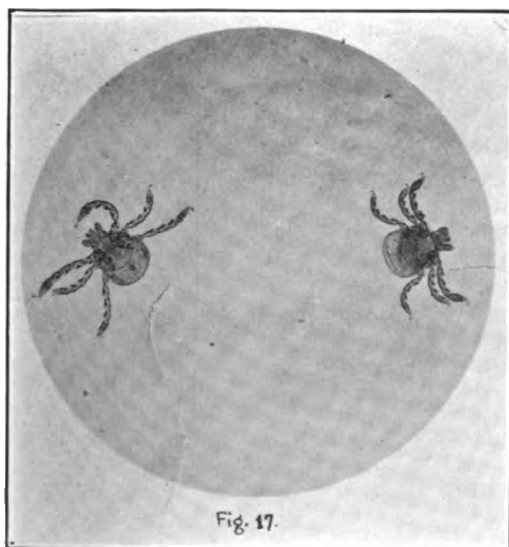
B. australis Fuller: Mandible in sheath, showing shagreen-like character of sheath near *a*. (1000 X.)



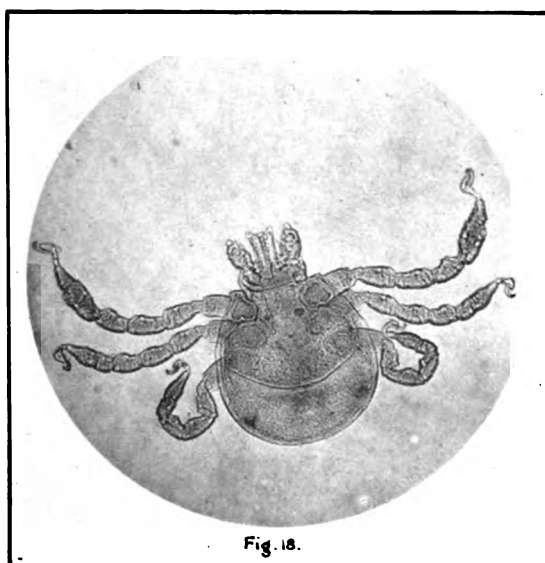
B. australis Fuller: Scutellum of female, showing antero-dorso-lateral projections at *b* and eyes at *a*. (19 X.)



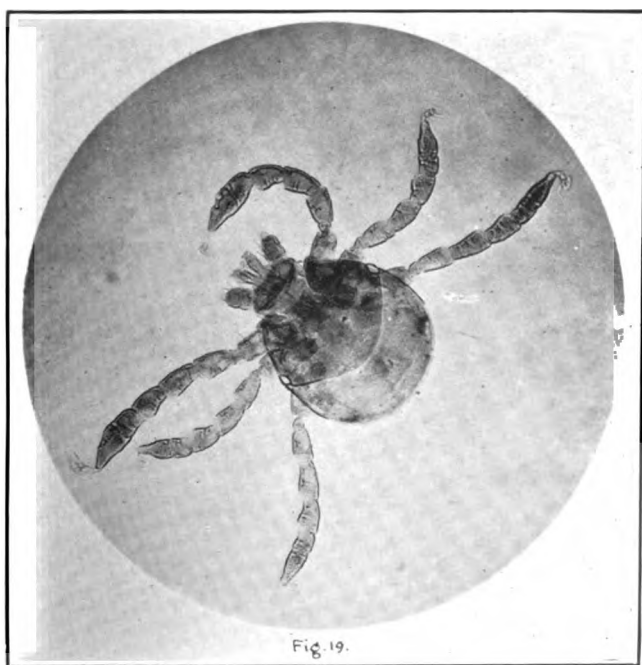
B. australis Fuller: Stigma of female, showing star-like structure. Slightly broken at upper left margin. (60 X.)



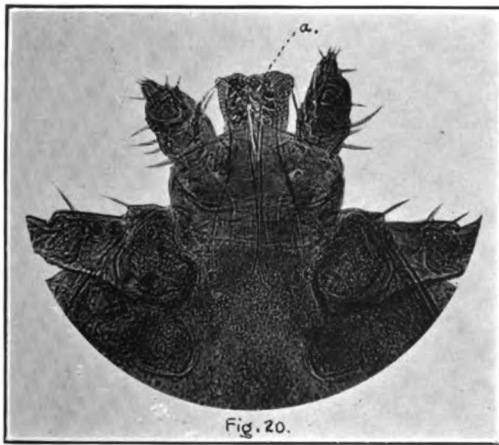
Boophilus australis Fuller: Young ticks, larvae, first hatched from eggs. (20 X.)



B. australis Fuller: Young tick, showing rostrum and claws of feet. (50 X.)



B. australis Fuller: Young tick, showing eyes and curved dorsal line. (50 X.)



B. australis Fuller: Rostrum of young tick, showing only two rows of ventral-paired denticles. (130 X.)

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BUREAU OF GOVERNMENT LABORATORIES.

BIOLOGICAL AND SERUM LABORATORIES.

REPORT ON BACILLUS VIOLACEOUS MANILÆ:
A PATHOGENIC MICROORGANISM.

By PAUL G. WOOLLEY, M. D.

MANILA:
BUREAU OF PUBLIC PRINTING.
1904.

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LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,

Manila, P. I., May 13, 1904.

SIR: I have the honor to transmit herewith a "Report on Bacillus Violaceus Manilæ, a Pathogenic Microörganism," by Dr. Paul G. Woolley, Assistant Director of the Serum Laboratory.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY,

Manila, P. I., May 12, 1904.

SIR: I have the honor to submit herewith and recommend for publication a "Report on Bacillus Violaceus Manilæ, a Pathogenic Microörganism," by Dr. Paul G. Woolley, at present Assistant Director of the Serum Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

DR. PAUL C. FREER,
Superintendent Bureau of Government Laboratories,
Manila, P. I.

REPORT ON BACILLUS VIOLACEUS MANILÆ: A PATHOGENIC MICROÖRGANISM.

By PAUL G. WOOLLEY, M. D.,
Assistant Director Serum Laboratory.

I can find no account in literature of a pathogenic organism similar to the one of which a description follows, but there are one or two bacilli which resemble it so closely in morphologic and cultural characteristics that it seems wisest to consider the present germ as simply a variety of these well known but at least usually nonpathogenic forms.

The group to which reference is made is the one, the individual members of which are discussed collectively by Migula (*System der Bakterien*, 1900, II, 939) under the title of *Pseudomonas violacea*, and which are separately described by other authors as *B. violaceus*, *B. violaceus berolinensis*, *B. violaceus lutetiensis*, *B. violaceus laurentius*, etc.

The organisms are described by these authors as bacilli, which are short or of medium length, sometimes somewhat bent, and with rounded ends. The measurements are about 1 by 0.65 μ , although some individuals may attain a length of 3 μ . In cultures on agar and potato, formations much resembling spores, but which are like these reproductive bodies in appearance only, are seen within the individual rods. These pseudospores are considered to be either plasma globules or vacuoles, and true spores have not been demonstrated. The rods are motile—sometimes, as in cultures kept at body temperature, quite actively so. They possess a single polar flagellum. Deep colonies in gelatin have an irregular, rounded appearance, with projecting filaments. On the surface the colonies form small depressions, in which blue-gray masses of bacilli are seen. The color gradually becomes more intense, and the straggling filaments grow out into the surrounding medium. On agar the growth is blackish violet. Blood serum is liquefied—peptonized. On potato the growth spreads over the surface of the

medium and forms a rich pigment, which does not stain the whole fragment of the vegetable but only that portion immediately beneath the growth. Broth is clouded, and a dirty white sediment falls to the bottom of the tube and takes on a pale violet color. No pellicle is formed on the surface. The organisms grow fast at room temperature, but more slowly than most water bacteria, and they do not grow beneath the isinglass. They are most readily stained with hot carbol-fuchsin, and are decolorized by Gram's method.

B. violaceus manilæ corresponds with the above description in some details, but in some it does not. The organism is a short rod, measuring approximately 0.5 by 1 to 1.5 μ . Occasional rods are even longer. It stains with the usual aniline dyes, best perhaps with carbol-thionin, or with carbol-fuchsin or gentian-violet diluted five times with water. It is not stained by Gram's method, and is not "acid fast." When well stained by any of the above-mentioned solutions, it appears as a short, thin bacillus, very frequently slightly bent, and with rounded ends. It does not, as a rule, stain uniformly, and may show one or more clear spaces which are not tinted and which appear remarkably like spores. These clear spaces can not be stained by the usual methods used in coloring spores. The organisms are generally single, sometimes in pairs, occasionally arranged in short chains of three or four individuals. They are motile, usually sluggishly turning and twisting, but frequently single rods may be seen to cross the field of the microscope with a more rapid, wriggling motion. Each organism possesses one polar flagellum. In rare instances two flagella may be distinguished springing from the same pole of the bacillus.

On agar plates, within twenty-four hours at 37° C., colonies appear as small, round, violet-gray spots. These slowly enlarge and become deeper in color. The maximum depth of color is attained in from forty-eight to seventy-two hours. As the colonies grow, their margins become slightly irregular and with a rather indefinite concentric arrangement of layers, which are somewhat thicker toward their peripheries, with the result that the centers of the colonies have a less intense blue color than the edges. These masses of organisms are slightly tenacious, but after removal from the medium are readily dissociated in water. The growth on agar slants is similar to that on plates, extension being slow but rather even, resulting in a very slight crenation. The water of conden-

sation is clouded and bluish, with a blue sediment. In stab cultures in agar the growth is scant, and no pigment is formed below the surface.

Gelatin is liquefied slowly at the temperature of the ice box, the growth forming a funnel-shaped area with a cup-shaped upper portion. The sediment is bluish.

Bouillon is diffusely clouded. A delicate pellicle is formed, which, upon 1 per cent alkaline (to phenolphthaline) material, is but slightly pigmented, but which is better developed and bluer on a 1 per cent acid liquid. The sediment in 1 per cent acid bouillon is pale violet blue and rather viscid.

Dunham's peptone solution is also clouded, and a thin, pigmented pellicle is produced. A sediment is deposited in this medium which resembles that in broth but in which more pigment is present. The whole medium becomes diffusely bluish within forty-eight hours.

On potato, the growth extends over the whole surface of the medium and to the water of condensation. The pigment production is luxuriant. The superficial layers of all the solid media are stained by the soluble blue pigment. In sugar containing media—glucose and lactose—no gas is formed.

The reaction of milk, after seven days, is slightly acid, but there is no coagulation. However, the casein is peptonized, and the upper third of the tube becomes almost clear and faintly violet.

The organism thrives better and produces pigment somewhat more freely at 37° C., than at lower temperatures. It does not grow at all well and does not produce any pigment below the surface of a solid medium. It is almost an obligatory aërob. It is killed by boiling for one minute, by a temperature of 67° C. after an exposure of five minutes, and by a temperature of 57° C. after one hour. It does not form spores.

The pigment is soluble in alcohol, giving a deep, rich, violet solution. It is slightly soluble in water, barely dissolved by ether, and insoluble in chloroform.

The organism described above has been isolated from three carabaos, which died suddenly with such noticeable absence of clinical symptoms that acute hemorrhagic septicæmia was suspected. In two of these cases Dr. Jobling obtained cultures of this organism from the lymph glands and lungs, and in the third case the writer found it predominating in cultures from the same organs. Later on, in the press of work, the cultures from the first cases were lost,

and only those from the third were used in the following investigation. However, Dr. Jobling had injected his organism into guinea pigs, producing the same kind of lesions which I shall describe. The details of the first autopsies are meager. Little can be said, except that the prescapular glands resembled those to be described in connection with the third case.

Like the first ones, the third animal died suddenly. It had had no rise in temperature, as far as the records of the corral show, and symptoms of rinderpest were absent. On removing the hide none of the gross lesions of hemorrhagic septicæmia or surra were encountered, neither hemorrhages nor œdemas. The prescapular glands were enlarged and intensely injected, but neither showed true hemorrhages nor necrotic areas. There were a few small petecchiæ under the visceral pericardium and under the endocardium of the left ventricle. The lungs were not collapsed, but were for the most part crepitant. The surfaces of these organs were dotted with small, pale areas varying in size from that of a pinhead to a small hazelnut. These areas were firm and projected slightly above the surface of the pleura. They were not round, but irregularly stellate, and each was surrounded by an appreciable zone of congestion. On section they appeared granular and gray, and with no indication of caseation or suppuration. Other noncrepitant areas which occurred chiefly along the anterior margins of the lungs had much the appearance of red infarcts. They were dark red and raised above the general surface of the organ in which they occurred. On section they were dark and moist, simulating the stage of red hepatization in pneumonia. The lungs on section were generally pale and œdematous, and in their substance contained large numbers of the small miliary nodules similar to those seen upon their surface. The spleen appeared to be normal. The liver showed no macroscopic changes. The gall bladder was somewhat larger than normal, but the stomach showed no pathologic lesions visible to the naked eye, nor were any found in the trachea, pharynx, or tonsils. The kidneys were perhaps a trifle pale. The intestinal tract was normal.

Smears from the prescapular glands showed a few very small organisms, which appeared as diplococci or polar-stained bacilli, and a large number of somewhat larger rods; some of these irregularly stained, and others curved. Cover-glass preparations made

from the nodules of the lungs showed almost nothing but small bacilli, which stained unevenly. Smears from the heart showed no organisms.

Cultures from the blood gave no growth after some days, and those from the lung lesions produced a few colonies of a large thick bacillus, resembling *B. subtilis*, and other colonies in very much larger numbers (in one plate, these latter only), which developed a blue color after twenty-four hours at 37° C. and which were composed of the organisms which have been described. In cultures from the prescapular glands two organisms were demonstrated. One was identical with the chromogenic one isolated from the lungs; the other, a small coccus, arranged mostly in clumps, and which, after several days, produced a very faint yellow color. Both of these organisms—the coccus and the chromogenic bacillus—were studied carefully. One was in all probability a modified *Staphylococcus aureus*, which produced a minimum of pigment, coagulated milk very slowly with coincident reduction of the litmus, and which did not kill guinea pigs in reasonable doses, either when injected into the peritoneal cavity, or under the skin. The other has been described in its cultural and morphological character, and corresponds quite closely with *Bacillus violaceus* (Schröter), or *Pseudomonas violacea* (Migula). In none of the books to which we have access have I been able to find any reference to the pathogenicity of *Bacillus violaceus*. This makes the following experimental study of some interest:

One cubic centimeter of a forty-eight-hour-old culture from the lung of the carabao (No. 431) was injected under the skin of a guinea pig (No. 329). The animal became quite ill within the next twenty-four hours and a large semifluctuating mass appeared at the site of the inoculation. Coincidentally the temperature, which previously had been running between 36.5° and 38.4° C., rose to 39.8° C. and then dropped gradually until the animal died. Death occurred on the fifth day after infection.

At autopsy a large area of necrosis was found under the skin about the point of inoculation. This was surrounded by tissue in a state of coagulation necrosis, in which were occasional lacunæ filled with a dark, gelatinous material. There was no true pus and no hemorrhages appeared, although the adjacent tissues showed extensive congestion. The peritoneal cavity contained a small amount of a clear fluid. The lungs were the seat of large and small

hemorrhagic infarcts, the lower lobes of both sides being almost completely infarcted. The spleen was markedly enlarged, was very dark, soft, and mottled with miliary gray spots, resembling focal necroses. The liver was also thickly studded with similar areas. There were no macroscopic lesions in the heart or kidneys, though the latter were pale. The adrenals were markedly enlarged, their medullæ congested, and with small areas of necrosis in the cortices. The lymph glands of the axillæ and groins were enlarged and injected. Nothing abnormal was remarked in the intestines, stomach, or bladder.

Smears were made from the subcutaneous tissues at the site of the primary lesions from the lungs, spleen, liver, lymph glands, and heart. All save those from the heart showed numbers of rods morphologically identical with those which had been injected. The organism was recovered in pure cultures from the liver, peritoneal cavity, lungs, the primary lesion, and the heart.

A second guinea pig (No. 458) was inoculated intraperitoneally with one-half of a cubic centimeter of a bouillon culture obtained from the carabao. It survived the operation.

From the cultures obtained from the first guinea pig a second (No. 327) was inoculated with one-half cubic centimeter of an emulsion made with three loopfuls of a twenty-four-hour-old agar culture in 5 cubic centimeters of a normal salt solution. The animal died on the third day after inoculation. The anatomical picture was the same as that in the first guinea pig, except that in the lungs small miliary necroses were present in place of the infarcts noticed before. Pure cultures of the experimental organisms were obtained from the heart, liver, and the subcutaneous lesions.

With 1 cubic centimeter of an emulsion made with three loopfuls of the organisms obtained from this last animal and 5 cubic centimeters of salt solution, a rabbit (No. 474) was inoculated subdermally. It died within thirty-six hours. Autopsy showed a bloody fluid in the peritoneal cavity, a widespread hemorrhagic lesion at the site of inoculation with no suppuration, but with necroses in the liver. The organisms were recovered in pure culture from the peritoneal cavity, liver, and heart. At the same time a guinea pig, inoculated with an equal amount of the same material, died within twenty hours. The only macroscopic lesions were miliary abscesses of the liver. Cultures were obtained from the heart, liver, and peritoneal cavity.

A cat (No. 366) was inoculated under the skin of the belly with 1 cubic centimeter of an agar suspension of a culture obtained from guinea pig No. 327. The succeeding day a large semifluctuant mass surrounded the point of inoculation. The second day after infection this abscess was discharging externally by a sinus, the edges of which were ragged and about which the skin was semi-necrotic. Eventually all the skin about this first sinus sloughed, leaving an ulcer measuring 5 by 5 centimeters, whose base was on the subjacent muscles and whose edges were regular, indurated, and undermined. This ulcer gradually healed, and the animal showed a complete skin on the thirtieth day after inoculation. When recovery had been established, a second dose of a suspension of the organisms from No. 474 was introduced hypodermically. No lesion was produced. The serum from this animal taken at this time agglutinated the specific organisms in a dilution of 1 to 60 after about forty minutes, in dilution of 1 to 200 after about one hour and fifteen minutes.

Five rabbits were inoculated with different amounts of the organisms to show, if possible, what variations occurred in the lesions. The first (No. 454) received one-half loopful; the second (No. 455), one; the third (No. 436), two; the fourth (No. 435), three, and the fifth (No. 433), four, of a culture obtained from rabbit No. 474. All of these animals died—Nos. 454, 455, and 436 in three days, No. 435 in four days, and No. 433 in five days after inoculation. From all, the organism was recovered from the tissues and heart's blood, except in two cases (No. 435 and 436), in which the cultures from the heart were negative.

The progressive changes in the organs of these animals were noticeable and interesting. In animal No. 454 the subcutaneous jelly-like oedema was present; there were miliary abscesses in the liver, none in the spleen, none in the lungs, which were simply injected. In animal No. 455 there were a few nodules in the lungs and in the liver; the other organs appeared like those in animal No. 454. In animal No. 436 there were large abscesses in the lungs, and but a few small ones in the liver. Animal No. 435 showed similar lesions. In No. 433 there were a few very small abscesses in the liver and spleen, while the lungs were generally consolidated, showing comparatively large areas composed of collections of miliary abscesses. In other words, the lesions varied as the dose of the organisms, and while with small doses the liver was

more prone to be affected, with large ones the lungs were more prominently diseased. This may or may not be true for other animal species. At any rate, the principal feature of the pathogenic action of the bacillus is its necrotizing power.

A dog (No. 515) was inoculated subcutaneously with 1 cubic centimeter of an agar suspension of a culture from No. 474. Several days later a local lesion appeared, which resembled that produced in the cat, but which was less extensive and healed more rapidly. About three weeks afterwards this dog received 1 cubic centimeter of similar material in the femoral vein. Following this injection the animal was irritable, eating but little for a few days. On the third day, and continuing for four days, tremors were noticed in the head and limbs, the animal lying quietly without seeming to suffer. He subsequently became entirely well.

In the case of another dog (No. 516), in which the organisms were introduced into the trachea, no illness followed.

A calf was inoculated in the dewlap with 2 cubic centimeters of the same material which was used with the dogs. The day following inoculation there was a large, painful, edematous mass visible in the region of infection. This enlarged until it reached the size of a large fist, and then gradually disappeared.

Inoculation into the peritoneal cavity of 1 cubic centimeter of a forty-eight-hour-old agar culture suspended in salt solution caused the death of a rabbit (No. 492) within twelve hours. At autopsy there was nothing to be seen except evidence of an acute peritonitis. Cultures from heart and peritoneal cavity were positive and pure.

Intravenous inoculation of one-third of a cubic centimeter of a forty-eight-hour-old agar suspension killed a rabbit (No. 490) within ten hours. No lesions were visible to the naked eye. Cultures made from the heart, peritoneal cavity, and pleura showed the organisms in uncontaminated growths.

Feeding experiments were negative. Large quantities of virulent agar cultures were fed to two monkeys (Nos. 488 and 489) with no ill effects.

Experiments upon serum reactions were begun with a monkey (No. 468), which had received a dose of 1 cubic centimeter of an emulsion made from a twenty-four-hour-old agar culture of the organisms isolated from the carabao. The monkey did not succumb to this first subcutaneous injection, and four days later a second one was made with $1\frac{1}{2}$ cubic centimeters of an emulsion

made with a twenty-four hour culture of the organisms isolated from the second guinea pig (No. 327). The day following this second injection a small quantity of blood was withdrawn and its agglutinative powers tested. In dilutions of 1 to 20 a complete agglutination was present at the end of fifteen minutes. In 1 to 40 a complete reaction was given in half an hour. In a dilution of 1 to 200 the result was positive and complete in one hour.

Later this animal was inoculated subcutaneously with $1\frac{1}{2}$ cubic centimeters of an agar suspension made with a culture of the organisms recovered from animal No. 474. Two days afterwards a large slough appeared on the abdomen about the point of inoculation, and the animal was very ill. It was killed and the blood drained off into a sterile tube. The serum obtained from this blood was tested for its agglutinative and bactericidal powers. In a dilution of 1 to 200 agglutination was complete in twenty minutes, 1 to 400 in thirty minutes, 1 to 600 in forty-five minutes, 1 to 1,000 in fifty minutes, 1 to 2,000 incomplete after one hour, 1 to 4,000 was negative.

This experiment was repeated in small tubes, so that the reaction could be watched with the naked eyes. The tubes were allowed to stand overnight. The next day all the organisms were contained in a precipitate, except in the control tube in which the liquid was still cloudy. Cultures made from all these tubes gave luxuriant growths. No appreciable bactericidal action was present.

To determine whether or not a soluble toxin was produced, a four-day-old culture in peptone was filtered through a Pasteur-Chamberland bougie F. The filtrate was kept at 37° C. overnight and no growth occurred. The next day 5 cubic centimeters of the material was injected under the skin of a monkey (No. 509). Following this there was no sign of toxæmia, no rise of temperature, nor any sign of altered health in the animal.

After it had been proved that even very small amounts of the living organisms would cause death of small animals and at the same time produce the specific lesions without the appearance of any appreciable immunity, cultures were heated to 57° C. for one hour, and these dead cultures, suspended in normal salt solution, were used for injection.

This material was used subcutaneously in a guinea pig (No. 486) and a monkey (No. 411). In the case of the monkey one cubic centimeter of an agar suspension was used for the first injection.

There was no appearance of a reaction until the fifth day, when the temperature rose one degree above the normal one for the animal. The day following this reaction a second injection of the same amount of the same material was given. On the fourth day following, the blood was tested for its agglutinating powers. Complete agglutination was accomplished in dilutions of 1 to 400 in fifteen minutes. A third inoculation was then made with 0.75 cubic centimeter (intraperitoneal) and 0.75 cubic centimeter (subcutaneous) of the same material which had been used for the other inoculations, and four days later a fourth inoculation was made with $2\frac{1}{2}$ cubic centimeters subcutaneously. Two days after the last inoculation the monkey was found dead. At autopsy there was a small pocket of a bluish material at the site of the last inoculation. Cultures from this lesion, from the heart, liver, and spleen were entirely negative, and the media was perfectly sterile after five days. The serum taken from the heart at autopsy gave a perfect agglutination in a dilution of 1 to 1,000 in forty minutes. The guinea pig suffered no harm from the inoculations.

Later, using cultures of the organisms from animal No. 474, and which had been killed by heating to 70° C. for forty-six minutes, a dog was inoculated with a series of injections. He remained well throughout the time. However, after the third inoculation his blood agglutinated in a dilution of 1 to 20 only, after one hour.

The essential lesions produced in experimental animals are found at the site of inoculation, in the lungs, liver, lymph glands, and spleen.

At the site of inoculation, when infection has been caused subcutaneously, there is a wide area of necrosis with local and circumambient œdema resembling the lesions produced by the diphtheria bacillus. The whole area may undergo necrosis, become gangrenous, and slough away.

In the rabbits the œdema has been more marked than the necrosis. In the cat the necrosis occurred with but little œdema. The monkeys showed œdema as well as necrotic and gangrenous processes, as did the guinea pigs.

The lesions in the parenchymatous organs are miliary abscesses, which may show a suppurative stage. In the lungs and liver the surface of these abscesses may be covered with a fibrinous exudate. The bacilli are found in all the lesions.

The losses in stock due to infection with *B. violaceus manilæ* are

not extensive, and there seems to be little danger of an epidemic. In all our work we have seen but these three cases. Each was in a different herd, and these herds were widely separated from each other.

So far as treatment is concerned there is little to be said. All the animals have died so suddenly and unexpectedly that there was no time either for experiment or speculation on this subject.

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No. 16.—SEPTEMBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES
BIOLOGICAL LABORATORY

PROTECTIVE INOCULATION AGAINST
ASIATIC CHOLERA
(AN EXPERIMENTAL STUDY)

BY

RICHARD P. STRONG, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
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No. 16.—SEPTEMBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES
BIOLOGICAL LABORATORY

PROTECTIVE INOCULATION AGAINST ASIATIC CHOLERA

(AN EXPERIMENTAL STUDY)

BY

RICHARD P. STRONG, M. D.

MANILA
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1904

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LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., July 6, 1904.

SIR: I have the honor to transmit herewith and recommend for publication a paper entitled "Protective Inoculation Against Asiatic Cholera," an experimental study by Richard P. Strong, M. D., Director of the Biological Laboratory.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

PROTECTIVE INOCULATION AGAINST ASIATIC CHOLERA.

(AN EXPERIMENTAL STUDY.)

By RICHARD P. STRONG, M. D., *Director Biological Laboratory.*

The experimental work which forms the basis of this article was for the greater part performed during the spring of 1903 in the Institut für Infektionskrankheiten," Berlin (Prof. R. Koch, director, department of Prof. A. Wassermann). I wish here publicly to express my very grateful thanks to Professor Wassermann, under whose direction the research was first undertaken, for many suggestions and courtesies during the course of my studies. I also wish to express my gratitude to my colleague, Dr. P. C. Freer, for having kindly read the manuscript.

INTRODUCTION.

The epidemic of Asiatic cholera which has recently passed through these Islands has brought forcibly before us the particular difficulties encountered in combating and controlling a disease of this nature in a tropical country and among a partly uneducated people. Moreover, its history has demonstrated that it has not been possible to eradicate or even satisfactorily to control the malady in this city by ordinary hygienic methods—that is, by those measures solely directed toward the purification of the food and water supply of the infected districts. During the period in which the number of infected individuals was the greatest, it was shown by studies made in this Laboratory that in Manila at least the disease was not usually transmitted directly by water,¹ but probably more often by food infection. While cholera is not to be regarded, even in our present acceptance of the term, as a "contagious malady," undoubtedly in this epidemic the infection spread largely though as a rule indirectly, it is true, from

¹In other portions of the Archipelago the disease was certainly conveyed and spread by the water supply.

case to case. Thus, it was shown how, under the conditions existing here, an individual suffering with cholera or convalescent from it might frequently infect the food of several or many other healthy persons, and thus serve as the true means of continuing and spreading the disease. However, even at that period of the epidemic when general quarantine and isolation of each case discovered was carried out, the malady still continued to increase. While apparently almost every precaution practicable was taken by the Board of Health in regard to the furnishing of uninfected water and the prohibition of the sale of many fruits and other uncooked foods, and while also strenuous efforts were made in the isolation and treatment of the sick and the disinfection of their excreta, although the epidemic was partially held in check, nevertheless, as stated above, it spread, continued for nearly two years, and caused the death of 3,866 people in the city of Manila alone.¹ Hence, it was evident that, at least with a population of this character, it was not possible to prevent many individuals from coming into contact with and even ingesting the cholera organism. It therefore seemed advisable to immunize artificially and to protect by vaccination against the disease as many of this class of people as possible.

However, a few preliminary trials with Haffkine's method of protective inoculation showed the impracticability of using it in these Islands. First, because of the severe local and general reaction which it occasions when a good bactericidal immunity is obtained, the natives would not voluntarily submit to it; and second, on account both of this violent reaction and of the unsettled condition of the country, it was impracticable, or at any rate inadvisable, to make such vaccination compulsory. Lastly, while this inoculation gives rise to a bactericidal and agglutinative serum in the inoculated, the antitoxic value of such serum is probably very slight.

Because of the great importance of this question to the Government of these Islands, an experimental study was undertaken with the object of obtaining some practicable and efficacious form of protective inoculation against the disease. However, before proceeding directly to these studies, it will be appropriate to review briefly the investigations which have hitherto been made in this direction.

¹ In the provinces there were 90,745 deaths from Asiatic cholera reported by Maj. E. C. Carter, Commissioner of Public Health, during the epidemic.

A REVIEW OF THE METHODS OF PROTECTIVE INOCULATION PREVIOUSLY EMPLOYED.

The methods which have been employed for human protective inoculation against cholera are not numerous. Ferran, in 1885 in an epidemic which raged in Spain during that year, was the first to introduce the vaccination of human beings against the disease. He injected guinea pigs with small quantities of bouillon cultures which were inoculated directly from human cholera stools, and found that, in those animals which recovered, a certain immunity had been acquired, since, after a short time they resisted the injection of fatal doses of cholera spirilla. From these observations he decided to experiment upon human beings. His original method of vaccination was apparently for a time kept secret. It was supposed that eight drops of a bouillon culture of the cholera organisms, mixed with bile, were injected subcutaneously, and after an interval of from six to eight days, a second inoculation of 0.5 cubic centimeters of the same mixture was given; eight days later this second dose was repeated.

Subsequently, Ferran stated that his method consisted in using nothing more than a pure culture of the "comma bacillus" in bouillon, of which the dose was 1 cubic centimeter in each arm. Five days later revaccination was performed, the same amount being again injected. The subcutaneous introduction of the living cholera spirilla in this manner did not cause a general infection or give rise as a rule to alarming symptoms, though fever, malaise, lassitude, sometimes diarrhea, and always a considerable local reaction about the point of inoculation became manifest. It is said that about thirty thousand persons were vaccinated, but apparently no reliable statistics were obtained. Several government commissions were appointed to investigate Ferran's method, and their opinions in regard to its merits were usually unfavorable.¹ In general, it may be said that the inoculations, as they were carried on, were considered worthless. According to several of the reports the cultures employed were often not pure ones, nor was there any fixed virulence obtained for the organism used, and even the number of bacteria in a single injection varied greatly, so that an accurate regulation of the dose was not possible. The inoculations

¹Shakespeare's report spoke more favorably than the others of the results obtained.

finally became so disastrous that they had to be discontinued by the Spanish Government.

In 1888 Gamaleia reported that he was able to immunize guinea pigs and pigeons against fatal doses of the cholera spirillum by the injection of sterilized virulent cultures of this organism. He therefore suggested that this method be employed in human protective inoculation against the disease, emphasizing the advantages of such a chemical vaccine on account of the easy regulation of the dose as well as its sterility. Later he pointed out that after the destruction of the bacilli by heat, they produced only a moderate local reaction upon subcutaneous inoculation.

In spite of the bad results attending Ferran's work in Spain, Haffkine decided, as a result of animal experimentation, that successful active immunization in man could be obtained after Ferran's method, if it were rightly applied.

Haffkine's method of vaccination was as follows: An attenuated virus was first prepared by growing the cholera organism in flasks of bouillon at 39° C. while allowing a constant current of air to pass over the surface of the media. After this attenuation the germs were grown upon agar and carried from tube to tube. A virulent virus was prepared by inoculating a guinea pig intraperitoneally with cholera spirilla and then inoculating a second pig with the peritoneal exudate of the first, and so on through numerous animals until a very virulent culture was obtained. This was known as the fixed virus.

Haffkine maintained that the inoculation of guinea pigs with nonlethal doses of this fixed virus protected them not only against subsequent subcutaneous and intraperitoneal injections of cholera spirilla in lethal doses but also against the introduction of these organisms into the intestine or stomach after neutralization of the gastric juice.

The vaccination of human beings was performed in two stages. In the first 0.1 to 0.05 of a twenty-four hour agar tube of the attenuated culture suspended in bouillon was injected subcutaneously. In the second, performed from three to eight days after the first, the same amount of the virulent culture or "fixed virus" was inoculated. Haffkine states that on the injection of the attenuated culture only a slight local reaction was obtained, consisting merely of edema; and that no necrosis of the tissues took place. This

preliminary vaccination he also says modifies the reaction of the second.

Tamancheff showed that the addition of carbolic acid, in the proportion of 0.5 per cent, killed the organisms without, however, interfering with their immunizing properties. Local reaction and other toxic effects were also diminished, as shown in three human experiments. Haffkine also recommended carbolic acid in a 0.5 per cent solution for sterilizing the cultures after they have been grown upon agar.

Kolle first determined accurately that specific protective substances enter into the serum of human beings inoculated subcutaneously with cholera organisms; for, though Klemperer in 1892 made the same assertion, his experiments were not entirely conclusive. Kolle maintained that a single injection of the living cholera vibrios gave as good an immunity as when the inoculation was repeated, and further that the use of dead cultures produced about the same results as that of living ones. He vaccinated human beings and found that in those who had received a single inoculation of the killed vibrios the serum showed as good an immunity as in those which had been vaccinated several times with living organisms. Patients whose blood serum before inoculation showed a value of 0.75 and 0.6, ten days afterwards showed one of 0.003—that is, 0.003 grams of their serum protected guinea pigs against ten times the fatal dose of the cholera organism. After demonstrating that neither heat nor chloroform destroyed the value of the virus, he recommended the following method for human inoculation:

A well-grown agar culture containing about 20 milligrams of growth was suspended in 10 cubic centimeters of physiological salt solution and sterilized for a few minutes at 50° C.; 0.5 per cent phenol was added to the preparation without apparently interfering with the effectiveness of the virus. In vaccinating, 1 cubic centimeter, equal to 2 milligrams of the culture, was injected subcutaneously. Larger amounts, as high as one-fifth of a culture (4 milligrams), were occasionally employed by Kolle. Haffkine also sometimes used dead cultures, but thought that the living organism gave a greater degree of immunity and a more prolonged one.

The numerous observations made by Haffkine and others in India speak decidedly for the effectiveness of his own as well as of Kolle's vaccine and for the protection which is afforded

by them when properly applied. However, such methods will probably never come into general use, owing to the great discomfort and sometimes even serious results to which they give rise in the inoculated. A few hours after the subcutaneous injection of sufficient amounts of the living or killed virulent cholera spirilla into the human being, the local reaction becomes manifest. There is extreme infiltration in the vicinity of the injection and severe pain on pressure or even on the slightest movement of the inoculated extremity. The temperature rises to 39° or 40° C. There is faintness, general malaise, loss of appetite, and often severe headache and backache. After from one to three days the general and local symptoms usually begin to subside, although the local reaction may persist for a considerably longer time and may even go on to supuration. Indeed, it may be said that the subcutaneous injection of living or killed virulent cholera vibrios produces even more marked general symptoms and local reaction than the injection of either killed plague or typhoid bacilli. Therefore, it seems probable that, with such a vaccine, the reaction is so great that the method is not likely to be generally submitted to voluntarily.

Moreover, while there is no doubt that the subcutaneous inoculation of living or killed cholera spirilla gives rise to a bactericidal and agglutinative serum, it is very doubtful whether any very great toxic immunity is to be obtained by such injections. It must also be stated that when the bacterial bodies of the cholera organism are injected subcutaneously, in order to obtain a good immunity, a sufficient number to give rise to a severe local reaction must be introduced. The subcutaneous injection of small amounts of avirulent cultures of the cholera organism may be performed with the production of but slight discomfort.

The methods recited above are the only ones which have been extensively employed in the protective inoculation of man against Asiatic cholera, though a few isolated experiments in which other methods were used have been performed on human beings.

Thus, Klemperer in 1892 sought to obtain immunity in man and animals by the subcutaneous injection of the milk of goats which were immunized with the cholera organism. He maintained that the injection of 5 cubic centimeters of the milk of these animals produced such an immunity in man that 0.25 cubic centimeters of the blood serum of the inoculated individual was sufficient to protect guinea pigs against fatal doses of the cholera organism. Ketschner

also claimed similar results by the subcutaneous injection of the milk of goats, which animals had been immunized with cholera spirilla, but when the milk was administered through the mouth, no immunity was obtained. Later Klemperer inoculated himself subcutaneously with 3.6 cubic centimeters of a cholera culture sterilized by heating, and a short time afterwards his blood serum in doses of 0.25 cubic centimeters protected guinea pigs against doses of this organism fatal for the normal animals. Two weeks later he began to ingest killed bouillon cultures of the cholera spirilla, feeding himself one-half liter in divided doses for a period extending over twelve days. He then found that his blood serum in doses of 0.01 protected guinea pigs against lethal amounts of the cholera organism. Hence, it was twenty-five times stronger than before he began the feeding. No unfavorable symptoms from the ingestion of the spirilla were observed.

Sawtschenko and Sabolotny also performed feeding experiments upon themselves and their laboratory assistants. They used agar cultures in which the organisms were killed by successive heatings at 60° C. to 70° C. The bacteria were then suspended in normal saline solution, the liquid evaporated on a water bath and the organisms finally resuspended, sufficient carbolic acid being added to make a 0.5 per cent solution. After showing that their undiluted blood sera did not protect guinea pigs against a fatal dose of the cholera organism, they commenced to ingest the killed carbolized cultures, the experiment extending over a period of four weeks. During this time Sabolotny ingested an amount equal to 1.398 grams and Sawtschenko one equal to 0.838 gram of the dried bacteria. They stated that during their experiment slight symptoms of nervous depression and heaviness in the head were present. At the end of the time mentioned above their sera were collected and injected into guinea pigs in amounts of 1.5, 1.0, 0.5, 0.1, and 0.01 cubic centimeters. After three days the animals were all injected intraperitoneally with a twenty-four hours' agar culture, in amounts equal to 0.0006 of dried bacteria (twice the lethal dose). All these animals recovered, while one control animal without serum injected with 0.0003 gram of the dried cholera organisms died. Guinea pigs injected with 0.005 gram of the serum of Sawtschenko plus 0.006 gram of the dried bacteria also died. Death occurred also when the dose of agar emulsion was increased to 0.003 gram (ten

times the lethal dose), when even 0.5 cubic centimeters of the serum of each individual did not save the guinea pig.

In order to immunize themselves more completely, these authors continued their feeding experiments, Sabolotny finally ingesting 2.318 grams and Sawtschenko 1.758 grams of the dead bacteria. Then, in order to prove themselves immune to cholera infection, a few days after the last dose of the killed organisms, they neutralized their gastric juice with 100 cubic centimeters of a 1 per cent soda solution, and a little later ingested 0.1 cubic centimeter of a twenty-four hours' bouillon culture of the living cholera spirilla. No symptoms followed, although it was stated that cholera organisms were isolated from the stools in each case. This same culture injected into two rabbits in doses of 1 and 0.5 cubic centimeters caused the death of both animals.

While we now recognize that by feeding cultures of bacteria in large amounts a certain degree of immunity may be in some cases gradually developed, yet the method is tedious and very uncertain and has obtained no practical application. Moreover, in the case of toxines, with the exception of ricin, abrin, and certain snake poisons, the results are still more unfavorable for the production of toxic immunity by absorption through the normal gastric or intestinal mucosa.

Various other vaccines against the cholera spirillum, some of them chemically prepared, have also been described.

Brieger and Wassermann in 1892 prepared a virus by growing the cholera spirillum in bouillon prepared with the thymus glands of calves. The organisms were then killed by heating for fifteen minutes at 65° C. or for ten minutes at 80° C. and placed in the ice box for twenty-four hours. By the use of 4 cubic centimeters of this prophylactic in divided doses they were able to protect guinea pigs against three times the fatal dose for normal animals of the cholera vibrio.

Federoff obtained similar results with doses of 1 cubic centimeter of cultures grown in thymus bouillon for from seven to ten days at 37°C., sterilized by heating for fifteen minutes at 65°C., then allowed to stand in a dark room for twenty-four hours and finally mixed with an equal volume of glycerine.

In 1893 Wassermann prepared an extract of the organism in the following manner: One thousand cubic centimeters of a three to five day cholera bouillon culture were evaporated at 70° C. to 80° C.

to a sirupy consistency. The residue was treated with absolute alcohol and the heavy precipitate then filtered off and dried over sulphuric acid. 0.02 gram of this substance, when placed in the peritoneal cavity of a guinea pig, caused the death of the animal; but when 0.005 gram was injected the animal recovered and was found to be immune against ordinarily fatal intraperitoneal doses of the cholera organism.

A different procedure was recommended by Klebs who, after sterilizing cultures of the organism, filtered and concentrated them on a water bath. By precipitation with absolute alcohol the toxic substances were said to be separated out. These were then filtered and the filtrate used for experiments on immunization. Klebs called the preparation "anticholerin" and demonstrated its protective effect upon guinea pigs. He also recommended it for treatment in cases of Asiatic cholera.

Rosmainski in 1894 precipitated sterilized cultures with acetate of lead, removed the lead with oxalic acid, concentrated the filtrate, again precipitated with milk of lime, and finally sterilized the filtrate. This fluid was said to contain protective substances. When the cultures were precipitated with ammonium sulphate the precipitate dried and separation from the ammonium sulphate accomplished, with chloroform, the amorphous powder obtained was found also to possess immunizing properties.

Recently the Swiss Serum and Vaccine Institute of Berne has prepared a prophylactic against cholera obtained from the organism by the method which Lustig and Galeotti described for the preparation of their plague prophylactic. The agar cultures of the organism are dissolved in a 1 per cent caustic potash solution and then treated with 1 per cent acetic acid. The resulting precipitate is filtered off and washed to a neutral reaction and finally dried in a vacuum. Two milligrams of this nucleo-proteid dissolved in 1 cubic centimeter of a soda solution are recommended for the inoculation.

In 1894 Issaëff worked along the line of inducing immunity by increasing the natural resistance of the individual. He found in numerous experiments that different substances, such as tuberculin, nucleic acid, blood serum, bouillon, urine, and even physiologic salt solution, when injected intraperitoneally into animals, caused a transitory protection against infection with cholera spirilla. All of these substances possessed in common the faculty of calling forth

either a local phagocytosis in the peritoneal cavity or a general leucocytosis; the resistance declined as the number of leucocytes returned to normal. In the majority of cases, after four or five days this immunity had already begun to disappear. If, however, microorganisms were injected during this period, specific bactericidal substances entered into the blood and were demonstrable after from three to five months.

Buchner and Hahn sought to obtain immunizing substances from bacterial cells by special mechanical means. These authors triturated masses of the moist cholera bacteria mixed with infusorial earth and fine quartz sand. The organisms were subsequently subjected to a pressure of from four to five hundred atmospheres. The extract thus produced (the so-called cholera plasmin), when injected into guinea pigs in sufficient amounts, gave rise to the same results as the incorporation of the living cholera vibrios. The animals succumbed in from twelve to twenty-four hours, with a marked fall of body temperature. By a single injection of 0.5 cubic centimeters of this cholera plasmin, guinea pigs were protected against ten times the fatal dose of the living cholera organisms. This immunity was found to exist after from three to four months. The animals also showed an agglutinating serum. It may be added that typho-plasmin and tuberculo-plasmin were also prepared, which, it was maintained, also gave good results in the treatment of typhoid fever and tuberculosis.

Emerich and Loew showed that many bacteria, not only in the animal body but also in cultures, secrete enzymes, which in sufficient concentration are able to dissolve the organism producing them. They studied the sediment of old bouillon pyocyaneus cultures and found that most of the organisms were dissolved. In earlier cultures they observed that agglutination preceded this process and could be traced back to the saturation of the bacterial membrane with the enzyme. The pyocyaneus enzyme dissolved not only *Bacillus pyocyaneus*, but also cholera spirilla, anthrax, diphtheria and plague bacilli, as well as *Staphylococcus pyogenes aureus* and *Streptococcus pyogenes*. These authors maintained that natural immunity in man is based upon the presence of bacteriolytic enzymes in the blood, which possibly originate from the bacteria of the intestines. They designated these ferments as "nukleasen" and proposed the terms "pyocyanase" and "cholerae," respectively. For the production of these enzymes, a special culture medium contain-

ing asparagin, peptone, dicalcium phosphate, sodium acetate, chloride of sodium, and magnesium sulphate, was employed. After the growth of the bacteria the liquid medium was neutralized, filtered and evaporated at 25°C. to 30°C. until reaching one-tenth of its original volume, after which it was dialyzed. By the injection of the "pyocyanase" thus obtained animals could be successfully immunized against the injection of various bacteria, among them, the organisms of cholera, anthrax and plague. Rabbits received 12 cubic centimeters intravenously and 7 cubic centimeters subcutaneously without any ill effect and were fully protected.

Other authors, including Dietrich, were not able entirely to confirm this work by experimental investigation, though the damaging effect of "pyocyanase" upon anthrax bacilli, both in vitro and in the animal body, has been confirmed by Tavernari, Krause, and others.

Behring and Ransom, as well as Metchnikoff, Roux, and Taurelli-Salimbeni, showed the possibility of producing a soluble poison from a highly virulent cholera culture. Behring and Ransom obtained a toxine by the filtration of cultures, of which one-fourth cubic centimeter was said to kill guinea pigs of 300 grams' weight in eighteen hours, with all the characteristic appearances of a cholera infection. Metchnikoff and Roux found that by growing the organism in collodion sacs in the abdominal cavity of guinea pigs and afterwards upon a special medium containing gelatin mixed with serum a soluble toxine had been produced, which killed guinea pigs when administered subcutaneously in amounts of one-third cubic centimeter per 100 grams' weight. Experiments in the immunization of animals, especially of horses, were made. After six months' treatment a horse furnished a serum, 1 cubic centimeter of which had the power of neutralizing four times the fatal dose of the cholera poison. It was maintained that this serum was protective for animals not only against the cholera toxine but also against the injection of living vibrios and even against infection by way of the stomach. Practical use of these toxins for protective inoculation in man has not as yet been made.

Passive immunization against Asiatic cholera by the use of anti-toxic or bactericidal sera need not be discussed here, as it is obvious that from the standpoint alone of the brief immunity which they confer, the use of either for a practical prophylactic would not be satisfactory.

Finally Besredka performed experiments upon animals and a single inoculation upon himself with cultures treated in the following manner: The organisms were grown upon agar for twenty-four hours and suspended in normal saline solution, after which enough immune serum was added to cause complete agglutination. The mixture was allowed to stand for twelve hours, during which time the amboceptors and uniceptors of the serum became fixed to the bacteria. The clear fluid was then poured off and the agglutinated organisms freed from the excess of serum containing unbound amboceptors by repeated washings with normal saline solution. The residue, consisting of organisms plus the immune bodies, was heated to 56° C. for one hour and then injected subcutaneously. In the experiment which Besredka performed upon himself the plague bacillus was the organism employed, but similar results were obtained in animals in which the cholera spirillum was treated by the same method. He maintained that in this manner immediate immunity was conferred, which lasted for at least five to six months, and that no local or general symptoms followed the injection. He attributed the lack of a general reaction in his own case to the non-toxicity of the vaccine, and the absence of a local reaction he thought was probably due to the fact that, when the immune body was fixed to the bacilli, the latter became the prey of the phagocytes almost instantly.

He stated that the early appearance of the immunity may be explained in two ways, both according to the theory of Metchnikoff. He first refers to the idea that the immune body carried by the bacteria becomes free in the animal organism and thus acts as a preventive serum, that is to say, favors phagocytosis of the organisms introduced. Thus the immunity of the animal is assured in the beginning. He is, however, inclined to discard this explanation, since in the case of plague it required a period of forty-eight hours before the immune body manifested its preventive action. He therefore believed the immune body to have no other function than that of increasing and stimulating the work of the phagocytes in such a manner as to enable them to accomplish their action in a shorter time than would be possible in its absence. In this manner the time necessary to give rise to active immunity would be notably shortened.

It is obvious that another theoretical explanation can be given for the results of Besredka's experiments. Thus, it is clear that

in his prophylactic at the time of injection the haptophore groups of the bacteria were not all saturated with amboceptors and hence were capable of exerting certain immunizing power. Evidently, however, such power must be reduced below that possessed by the same bacteria (without serum) with no bound haptophore groups. The amboceptors which were introduced united to the bacteria could of course theoretically, after the destruction of the microörganisms in the body, exert a passive immunizing effect. However, the number of amboceptors injected in this manner would be less than that introduced in a moderate dose of immune serum alone.

From this brief review, therefore, we are apparently justified in drawing the conclusion that as yet no satisfactory form of human protective inoculation against cholera, effective as well as practical, has been established, since those prophylactics, which give rise to the necessary immunity produce too severe a local reaction, while no other, causing only a mild local reaction, has been shown to be equally or sufficiently protective. However, of the methods which have been employed, those of Haffkine and Kolle promise the best results.

I shall now refer to my experimental work upon this subject.

DESCRIPTION OF THE CULTURES EMPLOYED.

In my search for a practical vaccine, I first studied the local reaction and other toxic effects produced in animals after the injection both of a very virulent cholera culture and of one which, through cultivation on artificial media for a long period of time, had lost most of its virulence. The effects of the killed as well as of the living organisms were also studied with each culture. These two stems, for the sake of brevity, will be referred to in this article as "virulent" and "avirulent."

The avirulent organism was obtained through the kindness of Professor Wassermann. It had been isolated by R. Pfeiffer in the epidemic of cholera which occurred in Hamburg in 1894, and for nine years was preserved on artificial media in the laboratory and from time to time passed through animals. During the past year, however, the strain employed in these experiments had only been grown on artificial media and not inoculated into animals. Its growth on all culture media was typical for *Spirillum cholerae asiaticæ*, including the production of indol in proper peptone solution.

demonstrable by the addition of nitrite-free sulphuric acid. In its morphological characteristics it was not typical, in that short commas were seldom observed, but instead rather long, thread-like forms predominated; also its motility was to a great extent, though not entirely, lost. However, it was agglutinated by a standard cholera immune serum in even higher dilutions than is another genuine cholera strain; and there is no doubt whatever of its representing a true though attenuated type of *Spirillum cholerae asiaticæ*.

The virulent organism was isolated by Professor Kolle in Jaffa during the recent epidemic of cholera in that place. It reacted in all media in a perfectly typical way, and its morphology and motility were also characteristic of the genuine cholera organism. It is agglutinated in high dilutions by the same cholera immune serum, though not in so great ones as the avirulent strain.







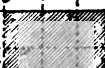


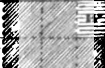


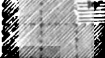


Some time was spent in accurately standardizing these cultures, and the minimal lethal dose for guinea pigs of 250 grams' weight was carefully determined. After numerous passages of "virulent" through animals, a lethal dose of 0.1 of a standard (2 mg.) *oese*¹ of a twenty-hour agar culture was reached. Such a dose of "virulent," when suspended in 1 cubic centimeter of an 0.85 per cent sodium chloride solution and injected intraperitoneally into a guinea pig of 250 grams' weight, regularly caused death within twenty-four hours. With "avirulent," on the other hand, one and one-half standard *oesen* of a twenty-hour agar culture, when injected intraperitoneally, were required to produce death within the same time in such an animal. The former strain, therefore, may be said to possess fifteen times the virulence of the latter. Throughout the course of the work this relationship between the organisms has been carefully preserved and continually tested by animal inoculation. As the virulence of cholera spirilla grown on laboratory media changes in a few days, it is necessary to make daily animal inoculations in the case of the virulent strain and always to use the same generation of the stem. With the avirulent culture considerable care was also necessary to keep its virulence fixed.


INOCULATIONS WITH LIVING CHOLERA ORGANISMS.

It soon became evident that the local reaction upon the tissues after the subcutaneous injection of "avirulent" was much less

¹ This standard *oese* was employed throughout the work.

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Rabbit	Inoculated with	Agglutination				
No.	$\frac{1}{2}$ Oese intraven.	Organism	1:50	1:100	1:200	Control Animals Without Serum.
V.	"Virulent"	"virulent"				6 control animals all neg; all dead in 24 hours.
		"avirulent"				
VI.	"Virulent"	"virulent"				9 control animals all neg; all dead in 24 hours.
		"avirulent"				
VII.	"Virulent"	Died after five days cultures from all				
VIII.	"Avirulent"	"virulent"				5 control animals all neg; all dead in 24 hours.
		"avirulent"				
IX.	"Avirulent"	"virulent"				8 control animals all neg; all dead in 24 hours.
		"avirulent"				
X.	"Avirulent"	"virulent"				7 control animals all neg; all dead in 24 hours.
		"avirulent"				

 - Complete liquid agglutination

 - Distinct agglutination
Overlying liquid

than after one with the "virulent" cholera spirillum. I therefore decided to determine the character of the serum which could be produced with this avirulent organism and to compare it with that produced by the virulent germ. Accordingly a series of six rabbits of an average weight of 1,500 grams was inoculated intravenously, three each with one-half *oese* of "virulent" and three each with one-half *oese* of "avirulent," the organisms in every instance being suspended in 1 cubic centimeter of bouillon. After eight days the rabbits were all killed by bleeding and the value of the serum in each case determined for agglutinative and bactericidal properties. It then became evident that the rabbits inoculated with the virulent culture always furnished better serum than those inoculated with the avirulent one, but that the value, in both agglutinative and bactericidal properties, of the serum from the animals treated with the former was in no case more than two and one-half times that of the serum furnished by the animals treated with the latter stem. The results of the experiments may be seen in detail in Table I.

TECHNIQUE EMPLOYED.

The technique of the agglutinative and bactericidal reactions employed throughout the work was as follows:

The reactions for agglutination were performed in the test tube. One *oese* of the living organism was thoroughly suspended in 1 cubic centimeter of an 0.85 per cent solution of sodium chloride. The amount of serum to be tested, suspended in 1 cubic centimeter of a similar saline solution was then added, the tube well shaken, and the mixture allowed to stand two hours at 37° C. In a complete agglutination it is understood that the liquid overlying the precipitated bacteria appears entirely clear. By a weak reaction we understand one in which there is a distinct agglutination with precipitation of numbers of the organisms, visible to the naked eye, but in which the supernatant fluid remains more or less cloudy.

The bactericidal reactions were performed in the abdominal cavity of guinea pigs according to the well-known method of R. Pfeiffer, a hypodermic syringe with a blunt-pointed needle being employed for the injections, care being taken to avoid any injury to the intestine during the inoculation. The dilutions of the serum were made in normal saline solutions. One cubic centimeter of the diluted serum was then added to 1 cubic centimeter of bouillon

containing 2 oesen of "virulent" in suspension, after which 1 cubic centimeter of the resulting mixture was injected into the peritoneal cavity of a guinea pig of 250 grams' weight (or a little less), the animal thus receiving ten times the fatal dose of the living organisms. A fresh guinea pig was of course used for each reaction. The experiment was controlled by microscopic examination of a drop of serum from the abdominal cavity, made immediately and again twenty minutes after the inoculation, and obtained by means of a capillary tube, and by the inoculation of control animals with ten times the fatal dose of "virulent" but without serum. The result to the animal after twenty-four hours, whether it was then living or dead, was regarded as the final test, though the condition of the organisms in the abdominal cavity after twenty minutes was always carefully noted.

CONTINUATION OF THE EXPERIMENTS WITH THE INOCULATION OF THE LIVING ORGANISMS.

As some outcome such as was obtained in the experiments given in Table I was not entirely unexpected, but as the results were somewhat at variance with the ideas of Haffkine and quite different from what R. Pfeiffer and Friedberger found upon the intravenous injection into rabbits of dead cholera spirilla of different degrees of virulence, it was decided to repeat them. Accordingly, a second series of animals was inoculated just as the first, and on the day of inoculation, as in the previous series, the virulence of the injected organisms was verified as fifteen to one. The result was practically the same, for at the end of eight days the examination of the sera showed that the virulent stem had in only one case given a serum of more than about two and one-fourth times the bactericidal value of that produced by the avirulent one. In this one case the avirulent serum was between one-fourth and one-fifth as strong. (See Table II.)

We shall not discuss here in detail the interpretation of these results or attempt to explain the discrepancy in immunity in comparison with the relation of virulence between the two stems. This will be done in another paper. It will be sufficient perhaps to state here that apparently from the results of these experiments with the intravenous injection of living organisms into rabbits in amounts of one-half oese we might assume that the immunity produced is not directly proportional to the virulence of the inoculated organisms.

<i>Rabbit</i>	<i>Inoculated with</i>	<i>Agglutinated</i>	
<i>No</i>	<i>1/2 O:sc intranen.</i>	<i>Organism</i>	<i>Control Animals Without Serum.</i>
		1 50 1 100 1 250 500	
194	Virulent	virulent avirulent	2 control animals; reactions neg; all dead in 24 hours.
195	Virulent	virulent avirulent	1 control animal; reaction neg; dead in 24 hours.
196	Virulent	virulent avirulent	2 control animals; reactions neg; all dead in 24 hours.
197	Avirulent	virulent avirulent	2 control animals; reactions neg; all dead in 24 hours.
198	Avirulent	virulent avirulent	2 control animals; reactions neg; all dead in 24 hours.
199	Avirulent	virulent avirulent	1 control animal; reaction neg; dead in 24 hours.

BIE

1890

1890

1890

1890



In the experiments performed with the subcutaneous injection of the avirulent organism, in both the living and the dead state, while the local reaction, as already mentioned, was much milder than with the use of the virulent germ, there was always considerable inflammation about the point of inoculation, and it soon became evident that it would be highly desirable, if possible, to do away entirely with the bacterial cells.

In this connection it was necessary to recall that probably a satisfactory protective against Asiatic cholera must contain substances which give rise to antitoxic as well as bactericidal bodies in the blood sera of the inoculated. Hence, a consideration of both these substances was necessary. Accordingly, the problem of their extraction from the bodies of the bacteria was next investigated.

It may be said by way of parenthesis that in this connection, on reviewing clinical experience with Asiatic cholera, I thought of the conditions under which the toxins are set free in the human body in cases of this disease. In epidemic cholera it is very evident that the symptoms usually develop very rapidly after they have once begun, and, indeed, usually within a few hours after their appearance, either death or a severe state of collapse has supervened. The cholera process can not be satisfactorily explained except as the effect of an organic poison, and it therefore appears not unlikely that in a very short space of time a considerable amount of toxic material is manufactured and liberated. Further, if cultures are prepared from the intestinal material made during the period of the most acute symptoms and compared with those taken forty-eight hours or a longer period after the entrance of the stage of collapse, it will be found that in the latter condition there is usually a remarkable reduction in the number of cholera spirilla and apparently a great increase in the other intestinal bacteria. If this clinical experience is compared with the observations made in the laboratory, namely, that in agar cultures of cholera spirilla the maximum growth at 37° C. is obtained in from twelve to twenty hours and that after this time a rapid death of the spirilla takes place, so that according to Gotschlich and Weigang, after two days only 10 per cent (at the maximum) and after three days only 1 per cent (at the maximum) of the organisms which were present at the end of the first twenty-four hours are still alive; it may be supposed that perhaps the stage of the most violent symptoms in human cholera may correspond with the period during which the rapid

dying off of the spirilla occurs. This would then correspond to the facts observed in the laboratory cultures, and it might be presumed that it is at the time of death of the spirilla that the largest amount of toxine is set free.

It may be difficult to establish the correctness of such an hypothesis; but when one considers the work which has been performed in the laboratory in regard to the chemistry of the cholera vibrio, the facts are certainly suggestive of such a possibility. Since, moreover, this subject has a direct bearing upon the extraction of the immunizing properties of the organism, it will here be appropriate briefly to refer to these experiments and to examine more closely into the chemistry of this spirillum.

THE CHOLERA TOXINE.

Since the publication of the first article of R. Koch, in which it was held that the cholera paroxysm, and especially the algid state, was to be regarded as a specific intoxication, due to the absorption from the intestine of the metabolic products of the cholera organism, many authors have attempted to produce experimental proof of the existence and to isolate this specific toxine. Hueppe was one of the first to investigate this question. He and his pupils believed the toxine to be formed in the intestinal tract under nearly anærobic conditions, and attempted to reproduce experimentally the relations which exist in the human intestine. Accordingly, fresh hen's eggs were inoculated with the cholera vibrio. This organism was said to give rise to hydrogen sulphide produced from the proteid material after using up the oxygen present, thus bringing about anærobic conditions. With such relations Hueppe maintained that the specific toxine was formed in large amounts.

However, it has been shown subsequently by several authors (among them Doenitz and Zenthoffer) that the cholera organism in sterile egg culture media neither produces sulphuretted hydrogen nor, indeed, multiplies to any great extent under anærobic conditions; so that one is forced to the conclusion that Hueppe's media was not free from other microorganisms. The toxine which he obtained was probably one resulting from putrefaction due to anærobic bacteria. Scholl and Gruber, from cultures on eggs, also obtained a similar toxic peptone, which the latter was able to precipitate with alcohol. The work of Nicati and Rietsch, Pouchet, Villiers, Cantani, Kunz, Brieger and Fraenkel, Petri, Winter and

LeSage, Klebs, Gamaleia, Gruber and Sluyt, need only be referred to here. To-day it would appear that none of these authors were dealing with the specific cholera toxine alone.

R. Pfeiffer studied the filtration of old and young bouillon cultures of the cholera organism. He found that the filtrate of cultures from one to five days old possessed no poisonous action after injection into animals. Only old cultures which had grown for many weeks or months contained a soluble poison, which, upon filtration, caused the death of the animals inoculated. These poisonous materials, however, behaved in a manner entirely similar to that of the basic alkaloidal bodies, to which the name of ptomaines has been given. Their action became manifest in a very brief period of time and in a manner entirely different from that of the cholera poison. Further investigation, in which immunization was attempted with such products, made it certain that these dissolved substances which were contained in old cholera cultures had nothing to do with the primary and secondary cholera toxins. They are basic bodies which may be found in many old cultures of bacteria and are without any specific significance. However, R. Pfeiffer was able to show that in fresh agar cultures of cholera bacteria the bacterial cells contained a powerful toxic substance. If such cultures were carefully killed through short contact with chloroform vapor, or by heating at 65° C. for one hour and were then injected intraperitoneally in small quantities into guinea pigs, death resulted, even though such cultures were sterile. Ten milligrams of an eighteen-hour culture, which was exposed for ten minutes to the action of chloroform vapor, caused the death of a guinea pig of 200 grams' weight with all the symptoms of a true cholera intoxication. This intracellular poison showed considerable instability. According to Pfeiffer and Wassermann, through treatment with various chemicals—absolute alcohol, concentrated solutions of neutral salts, etc.—or by boiling or prolonged heating at 60° C., a change from the primary to the secondary cholera poison takes place. Therefore, in cultures which are killed at high temperatures, a less toxic effect may be expected than in those killed at lower ones, as the latter contain only the primary poison. The action of the primary toxine, from a physiological standpoint also, is different from that of the secondary.

The earlier work of Pfeiffer was performed with the organism known as "cholera Massowah," which is now known not to be a

genuine cholera spirillum. However, his work has been confirmed for the latter organism by numerous authors, among them Kolle and Wassermann, and at present there is apparently but little doubt that the true cholera toxine exists as a constituent element of the bacterial cell. However, up to the present time we have not been successful in obtaining it in a pure state. This poison apparently becomes soluble only through the disintegration of the bodies of the bacteria. In all fluid cultures the growth of the vibrios is after a time prevented through plasmolysis, plasmolysis, or digestion, and the bacteria die. Thus, the soluble toxine is set free, but in these cultures it seems to be very unstable and is soon destroyed.

On the other hand, other observers, principally Metchnikoff, Roux, and Taurelli-Salimbeni, all working together, show that the living cholera germ produces a soluble diffusible toxine. These authors base their claims upon the following experiments:

A small, sterilized collodion sac, of a capacity of three or four cubic centimeters, was half filled with peptone solution or nutrient bouillon, inoculated with the cholera spirillum, closed and placed in the abdominal cavity of a guinea pig. Another guinea pig received a similar sac containing an emulsion of one and one-half gelatin cultures of cholera bacteria suspended in peptone solution and killed by chloroform, while a sac containing only peptone solution was placed in a third animal. The last animal remained unaffected. The one which received the emulsion of dead bacteria showed a slight elevation of temperature and emaciation, while the animals which had received the living organisms died in from three to five days with all the appearances of cholera intoxication. The collodion sac in these animals still showed motile spirilla.

In order to produce this soluble toxine in artificial media, a highly virulent organism was grown in similar sacs in the abdominal cavity of a guinea pig. In this way a culture was obtained, one-one hundred and sixtieth cubic centimeter of which sufficed to kill guinea pigs. This organism was then grown in a culture medium consisting of 2 per cent gelatine, 2 per cent peptone, and 1 per cent sodium chloride with the addition of fresh guinea pig serum from another sac. Cultures from this medium after three or four days, when filtered, killed guinea pigs in from sixteen to twenty-four hours, on being administered in amounts of one-third cubic centimeter per 100 grams of body weight. The toxine thus obtained

was not materially changed on being boiled, but lost its toxicity on contact with the air and on exposure to light. With such a toxine it was maintained that a highly effective antitoxic serum could be produced in animals. Attempts made by some other competent observers to repeat these experiments have not as yet been successful.

It seems that there are objections to some of the conclusions of Metchnikoff and Roux. In the first place results of experiments made with collodion sacs it would appear are not entirely confirmatory of their ideas. As has already been intimated, the organisms confined in a collodion sac, in the abdominal cavity of an animal, would after twenty-four hours die in large numbers, and through plasmolysis and disintegration the toxine would be set free from the bacterial cells. The living organisms remaining would later give rise to additional toxine in the same way, and thus the death of the animal would eventually result. In the case in which the dead organisms were inoculated in the sacs, there was, as stated by these authors, an elevation of the temperature for several days and emaciation. The amount of cholera toxine present was obviously not sufficient to bring about the death of the animal.

To-day no one would suppose that the same virulence is to be expected from the dead organisms as from the living ones, and particularly would this be true in a closed collodion sac. With the living organisms there would be many successive generations from which additional amounts of toxine would be furnished. It is stated that at the conclusion of the experiments made by these authors living vibrios were still present. Hence, the total amount of toxine set free would be many times larger than that from one generation of the killed germ. Moreover, it is not improbable that with organisms killed by chloroform and placed in collodion sacs, the intracellular toxine is not likely to be entirely given up, unless some further disintegration of the cells takes place. In other words, such conditions are not favorable to digestion and plasmolysis, a point which will be referred to in a later paper.

It is difficult to confront such evidence as Buchner brings forth in his work on cholera plasmin, in which the toxine was extracted by grinding and pressing the bacteria. The following experiments, which may easily be performed, also seem very difficult to explain on any other assumption than that the toxine exists in the body of the organism:

Two, eighteen-hour agar cultures are taken and the growth of each suspended in sterile normal saline solution. No. 1 is filtered through a Reichel candle and the filtrate is injected into guinea pigs in varying amounts. It is then seen that this filtrate possesses very little toxic power. On the other hand, if what remains on the filter be injected, even though the organisms are killed before injection, the animal dies with all the symptoms of cholera intoxication. Evidently the bacteria contain the toxine.

If the second culture is carefully killed by heating to 60° C. for a brief period and the bacteria are allowed to digest themselves by their own ferments for two or three days, ground, pressed in a hydraulic press, and then filtered off, the filtrate obtained from these killed and digested organisms, when injected into animals, shows toxic properties. Regarding the so-called toxins of Behring and Ramson, it seems likely, as Pfeiffer has remarked, that the cholera toxine which they have written of does not represent the primary poison, but rather secondary toxins and alkaloids which originate in old cultures and from which, indeed, Brieger has obtained cadaverin. In still older cultures he found putrescin, cholin, methylguanidin, and other toxic substances.

In connection with the subject of toxine production it may be appropriate to refer to the investigations of Kraus. This author performed his work with an organism designated as *Vibrio Naskin*. It is certain that this organism was not a genuine *Spirillum cholerae asiaticæ*, for in a cholera immune serum which agglutinated several strains of the cholera spirillum in dilutions of 1:20,000 *Vibrio Naskin* was not agglutinated in dilutions higher than 1:400, and furthermore, the serum obtained with *Vibrio Naskin* agglutinated it in dilutions of 1:800, but did not affect the cholera organism. *Vibrio Naskin* also produced a hemolysine which was not neutralized by a cholera-immune serum, but was by an anti-*Vibrio Naskin* serum. Moreover, the precipitines of the two organisms were not identical. From this vibrio (Naskin) Kraus was able to obtain a powerful toxic substance, which caused death in rabbits, guinea pigs, and other animals. The filtrates of the bouillon cultures were also toxic. By heating to 50° C. the poisonous properties were destroyed. This toxine could not be identified with the one obtained from the cholera spirillum by Metchnikoff. However, it is interesting to note the production of a soluble toxine in a vibrio of this nature and to call attention

to the caution that must be exercised in the recognition of a cholera spirillum to be used in experiments relating to toxine formation.

From this review it is apparent that as yet no substantial evidence has been brought forth to show that the cholera spirillum produces, as do the diphtheria and the tetanus bacilli, a powerful soluble toxine. Even in fresh cholera bouillon and peptone cultures (two or three days old) there are found numbers of dead bacteria, from which, through plasmolysis and digestion, the intracellular toxines are set free. The most reliable evidence points to the conclusion that this toxine exists as an integral part of the bacterial cell, and I believe that in the remarks which are to follow I shall be able to give further proof of this point.

THE FERMENTS OF THE ORGANISM.

The increased attention given to the importance of ferments in physiological and pathological processes, for example, in the self-digestion of tissues and cells which often results from the activity of certain intracellular ferments, led me to investigate the action of the enzymes of the cholera spirillum upon its own protoplasm, and the effects of the digestive products which are formed. The cholera vibrio, under certain conditions, produces at least four of these enzymes.

Bitter first demonstrated the diastatic action of this organism and found that it develops an acid in nutrient solutions containing starch paste. Fermi succeeded in obtaining this ferment in a pure condition, not only in the case of the cholera spirillum but also with several other varieties of microorganisms. He demonstrated that it is formed in culture media free from starch, but that, on the contrary, in those free from proteid it is not produced. A temperature of 60° C. destroys or markedly decreases the activity of the ferment.

The inverting ferments, as is known, are not frequently produced by bacteria; but Fermi and Montesano succeeded in demonstrating that the cholera vibrio, in either sugar or proteid-free media, sometimes, though very inconstantly, produces ferments of this class.

The cholera spirillum has been shown by Schoffer to produce a rennet-like ferment, which is similar in its action to the rennin of the cow's stomach. Fokker showed that the action of this ferment was destroyed by a temperature above 60° C.

It is, however, with the production of its peptonizing ferment that we are most concerned at present. Bitter first showed that

the liquefying of the gelatin in cultures of this organism is due to a real ferment action and that it occurs independently of the living bacterial cell. A cholera culture in which the organisms had been killed by heating to 60° C. still showed intense peptonizing action. This author was able to obtain this ferment in a pure condition by the following method:

Sixty-five per cent alcohol was added to gelatin which had been liquefied by the spirillum. In this manner the proteid, but not the ferment, was precipitated. After twenty-four hours the precipitate was removed by filtration and the ferment was precipitated from the filtrate by the addition of absolute alcohol. It was found that, when collected on a filter and dried, the ferment could be dissolved in an aqueous solution of thymol and its peptonizing properties demonstrated on gelatin tubes. Like similar ferments, it converts an indefinite amount of coagulated albumen into peptone, and it is more active in alkaline than in acid solutions; thus resembling trypsin more than pepsin. A small amount of acid prevents its action.

A noticeable property of these peptonizing ferments in general is their great resistance to dry heat. Thus, for example, the ferment of *Vibrio Finkler-Prior* is said to resist heating for ten minutes at from 120° C. to 140° C. However, they are less resistant when subjected to moist heat, the same ferment then becoming inactive at 70° C. Damaging influences, such as light or poisons, which either kill the bacteria or prevent their growth, also affect the action of these enzymes, though sometimes they are more resistant toward certain chemical substances than the bacteria or even the spores of the latter. These ferments can digest and sometimes peptonize not only gelatin but also coagulated serum, egg albumen, fibrin, and casein of milk.

We need only mention the work of Sommaruga in regard to the production of a fat-splitting ferment by the cholera spirillum, since there seems to be some doubt as to whether such a ferment is actually produced by this organism. It is apparently not formed in ordinary media.¹

¹ C. Oppenheimer (Die Fermente und ihre Wirkungen, 1903, p. 290) states that Carrière found a lipase in cholera bacilli, but it would appear that the tubercle bacillus was the organism from which Carrière isolated this ferment. (G. Carrière: Sur L'Existence d'un Ferment Soluble dans les Cultures de Bacilles de Koch, Comptes Rendus Societe de Biologie, vol. 53, p. 320, 1901.)

It may also be added here that Kraus and Clairmont have recently again called attention to the phenomenon first noticed by Koch, namely, the hemolytic action of cholera cultures.

The question arose then as to what might be the action of this peptonizing ferment of the cholera spirillum upon cultures of this organism. Here it is necessary once more to refer to the work of Gotschlich and Weigang, who, as mentioned above, found that in agar cultures after forty-eight hours only 7.43 per cent, and after sixty-eight hours only 0.8 per cent of the cholera spirilla which were present at the end of the first twenty-four hours in the same medium, were still alive. In one of their experiments with an agar culture kept at 37° C. for from fifteen to twenty hours, 10,000,000 individual organisms died; at the temperature of the ice box this rapid death of the bacteria did not result. Indeed, they noted that agar cultures which had been grown at 37° C. for twelve hours and then placed at the temperature of the ice box for twelve hours longer showed a greater number of organisms than did those which were kept continuously for twenty hours at 37° C. Conradi investigated this question further and concluded that this rapid destruction of the organisms resulted from the action of certain degenerative products formed autolytically within the cultures of the bacteria. He demonstrated that, if cultures in which further growth had been arrested, were placed in reed sacs, impervious to bacteria and the autolytic bactericidal substances then removed by dialysis, a new growth could be observed. He also explained Gotschlich and Weigang's results, which showed that the death of organisms kept at the temperature of the ice box is less rapid, by the demonstration that at this temperature the enzymes of the organism are not capable of exerting any marked chemical action.

PREPARATION OF THE PROPHYLACTIC.

After a consideration of the data given above and acting upon the supposition (1) that the cholera toxine exists as an integral part of the bacterial cell, and (2) that it is set free after the death of the organism and probably partly through the action of its own proteolytic enzyme, which is not destroyed at 60° C., I determined to find out whether the other immunizing substances (agglutinine and bacteriolysine) as well as the toxine could not be separated from the bodies of the bacteria by a process of autolytic digestion.

Accordingly cholera spirilla from stems known to possess good

peptonizing powers were placed in an aqueous solution, carefully killed by heating and digested at 37° C. It was then found that the cholera receptors were set free in the fluid in great abundance, a fact which after filtration was easily demonstrable, since such filtrates possessed the power of binding uni- and ambo-receptors (agglutinines and bacteriolysines) in a cholera-immune serum, as well as the ability, after injection into rabbits, of giving rise to the appearance of toxic symptoms, and in the case of the ultimate recovery of the animal, to the entrance of antitoxic, bactericidal, and agglutinative substances in the blood serum. Therefore, a filtrate prepared in this manner immediately recommended itself for trial as a prophylactic.

It was prepared in large quantities after the following manner: The surfaces of large flat-sided flasks (after the pattern of Kolle), filled with agar, were sprayed with twenty-hour bouillon cultures of the organism, and the flasks were then put aside for twenty hours in the incubator at 37° C.¹ After this period the growth was suspended in sterile water, removed from the surface of the agar, and the suspension then placed in a sterile flask at 60° C. for from one to twenty-four hours. The mixture was afterwards put aside in the incubator at 37° C. for from two to five days, and finally filtered through a Reichel candle.

In certain experiments the milky fluid overlying the sediment of the bacteria at the bottom of the flask was poured off and the latter crushed and subjected to hydraulic pressure of 600 atmospheres. Later the extracts from these pressed organisms and the original aqueous solution, previously decanted, were together under pressure passed through a Reichel or Berkefeld filter.

EXPERIMENTS WITH THE PROPHYLACTIC.

INTRAVENOUS INOCULATION.

Since the intravenous inoculation of four rabbits with from 2 to 3 cubic centimeters of a fluid obtained in this manner from the

¹ Cultures of twenty hours' duration were always used, on account of the rapid death of the organisms in older cultures. Fresh beef was employed in the preparation of the media. The agar at the time of use had an alkalinity of 1 per cent to phenolphthaleïn. This gave a sufficient alkalinity to the aqueous suspensions of the organisms to bring about a favorable action of the proteolytic ferment.

<i>Rabbit</i>	<i>Inoculated with</i>	<i>Agglutination Exp.</i>					
<i>No.</i>	<i>ICC. intraven.</i>	<i>Organism</i>	<i>Dilution</i>				<i>Control Animals Without Serum.</i>
			<i>1:50</i>	<i>1:100</i>	<i>1:200</i>	<i>1:3000</i>	
58	"Virulent" <i>Prophylactic I</i>	"virulent" "avirulent"					5 control animals; reactions neg.; all dead in 24 hours.
59	"Virulent" <i>Prophylactic I</i>	"virulent" "avirulent"				W	4 control animals; reactions neg.; all dead in 24 hours.
60	"Virulent" <i>Prophylactic I</i>	"virulent" "avirulent"				W	3 control animals; reactions neg.; all dead in 24 hours.
61	"Avirulent" <i>Prophylactic I</i>	"virulent" "avirulent"				W	4 control animals; reactions neg.; all dead in 24 hours.
62	"Avirulent" <i>Prophylactic I</i>	"virulent" "avirulent"			N	N	3 control animals; reactions neg.; all dead in 24 hours.
63	"Avirulent" <i>Prophylactic I</i>	"virulent" "avirulent"			N	N	3 control animals; reactions neg.; all dead in 24 hours.

virulent strain, in which, however, the organisms were heated for only one hour at 60° C., caused the death of all the animals and showed the extract to be powerfully toxic, it was thought advisable to attempt to weaken this toxic action by a more prolonged heating of the organism, in order that the bactericidal and agglutinative immunity might be studied in the inoculated animals. Accordingly, for the next series of rabbits, twenty-hour agar cultures of the organism were suspended in sterile water, placed at 60° C. for twenty-four hours, digested for two days at 37° C., and finally passed through a Reichel filter. For the sake of comparison, both stems, the virulent and the avirulent, were treated in this way, and the filtrates were labeled respectively "Virulent Prophylactic I" and "Avirulent Prophylactic I." One cubic centimeter of each filtrate represented the number of receptors obtained from 2 oesen of the living organisms. The rabbits were of about the same average weight (1,500 grams) as those used in the experiments of Table I. Each animal was inoculated intravenously with 1 cubic centimeter of the filtrate (equal to 2 oesen). After eight days they were all killed by bleeding and the bactericidal and agglutinative values of their blood sera were carefully determined. The results may be seen in Table III.

Thus we see from animals Nos. 58 and 60 that, with the intravenous injection into rabbits of 1 cubic centimeter of virulent prophylactic I, there were obtained sera showing an agglutinative value with the virulent stem of about 3.3 and 2.5 milligrams, and with the avirulent one of about 2.0 and 1.66 milligrams, and a bactericidal value against the former strain of 0.09 and 0.08 milligram. However, in the rabbits inoculated with the avirulent prophylactic the sera were not of nearly so great a value, showing an agglutinative worth of only about 10.0 to 3.3 milligrams, and a bactericidal one of 1.1 to 0.5 milligrams. Indeed, on comparing the sera of animals Nos. 58 and 60 with those of animals Nos. 62 and 63, we see that those of the two former possess about thirteen and fourteen times as great a bactericidal value as those of the two latter (0.09 and 0.08 milligram against 1.1 milligrams.) However, in the case of animals Nos. 59 and 61 this proportion was not maintained, the bactericidal value of animal No. 59 representing only three times that of animal No. 61.

This series of experiments suggested that by the use of this method of autolytic digestion a more favorable result, that is a bet-

ter bactericidal immunity, was to be obtained with the virulent organism, and that the immunity acquired was within certain limits directly dependent upon the virulence of the stem used in the preparation of the virus. The value of the sera obtained from the animals inoculated with the virulent prophylactic also offered encouragement for a more extensive trial of this method with certain modifications.

Experiments with prophylactic II.—Therefore another quantity of the prophylactic was prepared, some slight changes being introduced in the method. Twenty-hour agar cultures of the virulent organism were suspended in sterile distilled water and the suspension was then divided into three portions, each being placed in a separate sterile flask and kept at 60° C. for twenty-four hours. The first portion was allowed to digest for two days, and the second and third for five. All three were then filtered separately, after which the third was reheated at 60° C. for two hours. It is necessary to state that with the same amount of organisms, twice as much distilled water was used in preparing the suspension of the agar cultures in the case of prophylactic II as was employed in that of prophylactic I. Hence, 1 cubic centimeter of the former filtrate contained only the number of receptors obtained from 1 *oese* of the living organisms, so that it possessed only one-half the strength of prophylactic I.

Four rabbits were injected intravenously, each with 12 cubic centimeters of this prophylactic, animals Nos. 86 and 87 receiving the portions digested for only two days, animal No. 88 that digested for five days, and animal No. 89 that reheated for two hours at 60° C. after five days' digestion; each animal receiving the number of receptors obtained from the digestion of 12 *oesen* of the living organisms. After eight days the animals were, as usual, killed by bleeding and the values of their sera carefully estimated, as may be seen from Table IV.

The results obtained in this series of experiments gave still greater encouragement for the method and suggested that by a digestion of five days, more receptors were set free from the bacterial cells in an aqueous solution than by one of two days.¹ Animals Nos. 86 and 87, each receiving inoculations of the portions digested

¹ Further experimentation has shown that the best results are obtained with from three to five days' digestion. No better sera were produced with filtrates which had been subjected to digestion for a longer period.

<i>Rabbit</i>	<i>Inoculation</i>	
<i>No.</i>	<i>12 C. intrac. (1 c.c.)</i>	<i>Control Animals Without Serum</i>
<i>86</i>	<i>Virus Prophyl. digested</i>	<i>2 control animals; reactions neg.; all dead in 24 hours.</i>
<i>87</i>	<i>Virus Prophyl. digested</i>	<i>1 control animal; reaction neg.; dead in 24 hours.</i>
<i>88</i>	<i>Virus Prophyl. digested</i>	<i>2 control animals; reactions neg.; all dead in 24 hours.</i>
<i>89</i>	<i>Virus Prophyl. digested & reheated</i>	<i>1 control animal; reaction neg.; dead in 24 hours.</i>

<i>Rabbit</i>	<i>Inoculated with</i>	<i>Donor</i>	
<i>No</i>	<i>6 cc. intraven (1 cc. = 2 dose.)</i>	<i>5000</i>	<i>Control Animals Without Serum:</i>
<i>192</i>	<i>Virulent Prophylactic III.</i>	<i>N/D</i>	<i>6 control animals; reactions neg.; all dead in 24 hours.</i>
<i>193</i>	<i>Virulent Prophylactic III.</i>	<i>2</i>	<i>5 control animals; reactions neg.; all dead in 24 hours.</i>
<i>204</i>	<i>Avirulent Prophylactic III.</i>	<i>2</i>	<i>5 control animals; reactions neg.; all dead in 24 hours.</i>
<i>205</i>	<i>Avirulent Prophylactic III.</i>	<i>2</i>	<i>4 control animals; reactions neg.; all dead in 24 hours.</i>

<i>Results</i>	<i>Inoculated with</i>	<i>Aggl.</i>	
<i>No.</i>	<i>12 Cc. intraven. 1 c.c. = 1000.</i>	<i>Organism</i>	<i>Control Animals Without Serum.</i>
256	<i>"Virulent"</i> <i>Prophylactic IV.</i>	<i>"virulent"</i> <i>"avirulent"</i>	1 control animal; reaction neg., dead in 24 hours.
257	<i>"Virulent"</i> <i>Prophylactic IV.</i>	<i>"virulent"</i> <i>"avirulent"</i>	1 control animal, reaction neg., dead in 24 hours.
177	<i>"Avirulent"</i> <i>Prophylactic IV.</i>	<i>"virulent"</i> <i>"avirulent"</i>	1 control animal; reaction negative., dead within twenty four hours.
178	<i>"Avirulent"</i> <i>Prophylactic IV.</i>	<i>"virulent"</i> <i>"avirulent"</i>	

for two days, showed an agglutinative value of about 1.25 and 1.43 milligrams, and a bactericidal immunity of 0.05 milligram; while animal No. 88, inoculated with the portion digested for five days, showed an agglutinative value of 1.1 milligrams, and a bactericidal value of about 0.04 milligram. Experiments with animal No. 89 demonstrated that reheating at 60° C. for two hours had destroyed to some extent the agglutinative substances of the bacteria which was made apparent by an evident loss of agglutinine in the serum, the latter showing a value of only 1.6 milligrams. The substances giving rise to the bactericidal qualities were apparently but little affected by the second heating, since the bactericidal value of the serum of animal No. 89 was between 0.04 and 0.05 milligram, which was only a little poorer than that of animal No. 88 (0.04 milligram).

Experiments with prophylactics III and IV.—Further information being desired as to whether, with five days' digestion, more receptors are always set free in the fluid than after the action of this process for two days, two more portions of the prophylactic were prepared with both strains, one being digested for five and the other for two days at 37° C. In prophylactic III sufficient sterile water was used for the suspension of the organisms so that 1 cubic centimeter of it contained the number of receptors obtained from 2 oesen of the living organisms; while in prophylactic IV, 1 cubic centimeter of the suspension equaled the receptors from 1 oese. Hence, in the inoculation of the rabbits in which prophylactic IV was employed, double the amount of fluid was injected. A glance at Tables V and VI will explain these experiments. The rabbits which received prophylactic III, which had been digested for five days, all furnished better sera than those injected with the virus of corresponding virulence of prophylactic IV digested for only two days. The best serum obtained from the injection of virulent prophylactic IV was that of animal No. 256, which showed an agglutinative value of about 1.4 milligrams and a bactericidal one of 0.06 milligram; while the best obtained with prophylactic III was from animal No. 192, this one showing an agglutinative limit of 1 milligram and a bactericidal value of 0.04 milligrams. Comparing the experiments of animals Nos. 192 and 193 of Table V with those made with animals Nos. 88 and 89 of Table IV, it will be seen that the rabbits employed in the former case furnished a

slightly better serum. The only difference apparent in the virus, however, was that prophylactic II was not so concentrated as virulent prophylactic III. In other words, even though, as far as could be determined, corresponding amounts of the digested organisms in solution were injected, the animals receiving the more concentrated virus furnished a slightly better serum. However, the differences in value of the sera are so slight that unless constant they might be explained by natural variation in the animals used.

From the experiments of Tables III to VI, it seemed that sufficient data had been obtained in regard to securing a good agglutinative and bactericidal serum by means of a protective prepared after this method, and that its success for intravenous injections was assured.

SUBCUTANEOUS INOCULATION.

The next step, then, was to discover what degree of immunity could be obtained when the prophylactic was injected subcutaneously. Accordingly, with this end in view, experiments were made with both strains, the inoculations being performed subdermally in rabbits. Such a series of experiments is recorded in Table VII, from which it may be seen that after the subcutaneous injection of 5 cubic centimeters of the most favorable virus, sera were obtained with an agglutinative value of from 2.5 to 1.6 milligrams and a bactericidal value of from 0.14 to 0.1 milligram. These results were regarded as very favorable.

EXPERIMENTS WITH THE DRIED PROPHYLACTIC.

In several instances the prophylactic was evaporated in a vacuum at 38° C. and then pulverized, after which it was redissolved in normal saline solution, and injected into rabbits both intravenously and subcutaneously in varying amounts. While a plain loss in the potency of the prophylactic thus treated is evident from the experiments recorded in Table VIII, nevertheless a very good agglutinative serum and bactericidal immunity were obtained. Animal Nos. 167, inoculated intravenously with 10 milligrams of the powder obtained from virulent prophylactic II, produced, after eight days, a serum which agglutinated in dilutions of 10 milligrams, and showed a bactericidal reaction in dilutions of 0.25 milligram. Animal No. 187, inoculated subcutaneously with 5

<i>Rabbit</i>	<i>Inoculated</i>		
<i>No.</i>	<i>Subcutaneous with.</i>		<i>Control Animals Without Serum.</i>
399	5 c.c. Virulent Prophylactic V. 1 c.c. = 8 oese.	NR 100	1 control animal; reaction neg.; dead in 24 hours.
400	5 c.c. Virulent Prophylactic V.		1 control animal; reaction neg.; dead in 24 hours.
423	5 c.c. Virulent Prop lactic V. Preserved in 5% carbolic ac	N D	1 control animal; reaction neg.; dead in 24 hours
426	2 c.c. Virulent Prop lactic V. reheated to 60°c. for 30 min		1 control animal; reaction neg.; dead in 24 hours.
174	5 c.c. Avirulent Prophylactic V. 1 c.c. = 1 oese.		1 control animal; reaction neg.; dead in 24 hours.
184	5 c.c. Avirulent Prophylactic		1 control animal; reaction neg.; dead in 24 hours.
440	5 c.c. Virulent Prop lactic VI. in Chlor tone (1 c.c. = 8 oese)		

<i>Rabbit</i>	<i>Inoculated with</i>	
<i>No.</i>	<i>Dried Prophylactic intracoen.</i>	<i>Control Animals Without Serum</i>
167	10 Mgs. Virulent II.	2 control animals; reactions neg.; all dead in 24 hours.
168	3 Mgs. Virulent II.	2 control animals; reactions neg.; all dead in 24 hours.
167	(Subcutaneous) 5 Mgs. Virulent III.	2 control animals; reactions neg.; all dead in 24 hours.
169	10 Mgs. Avirulent II.	2 control animals; reactions neg.; all dead in 24 hours.
170	3 Mgs Avirulent II.	1 control animal; reaction neg.; dead in 24 hours.
175	(Subcutaneous) 5 Mgs Avirulent III.	1 control animal; reaction neg.; dead in 24 hours.

milligrams of the powder obtained from virulent prophylactic III, showed after one week a serum of a weak agglutinative value of 10 milligrams and a bactericidal one of 1.6 milligrams.

STUDY OF LOCAL AND GENERAL REACTION FOLLOWING INOCULATION OF THE PROPHYLACTIC.

Speaking in a general way in regard to the inoculations recorded in Tables III to VIII, it may be said that the animals apparently suffered very little from the injection of the prophylactic, even when very large amounts were employed. Usually they showed a rise of temperature of 1 or rarely 2 degrees during the thirty-six hours immediately following the inoculation. A number of rabbits were treated subcutaneously with each separate lot of the prophylactic, for the sole purpose of observing the local reaction. Only a portion of these were killed subsequently and the value of their sera determined. (See Table VII.) In those animals receiving the injection subcutaneously, the skin was first shaved and subsequently carefully examined in the vicinity of the point of inoculation for any local reaction that might have appeared. Even when large amounts of the virulent prophylactic (5 cubic centimeters) were used, no suppuration ever occurred, and, indeed, induration was very rarely observed. Usually after twenty-four hours there was no trace of a local reaction visible to the naked eye, and upon palpation no induration was evident. These animals also showed a slight and transitory rise of temperature of 1 or 2 degrees.

In regard to the retention of the immunity, it may be stated that several of the animals were killed from three to six months after the inoculation and that at this time an examination of their blood sera still demonstrated a high agglutinative and bactericidal reaction.

COMPARISON OF THE IMMUNITY PRODUCED BY THE VIR- ULENT AND THE AVIRULENT PROPHYLACTIC.

In comparing the immunity obtained by the use of the virulent and the avirulent prophylactic, we see that on the whole the results recorded in Table III (already referred to) are borne out. It will be recalled that in this table the ratio of bactericidal immunity between the animals treated with the virulent prophylactic and those treated with the avirulent one varied between about $3\frac{1}{2}$ to 1 and 12 to 1. In Table V the sera obtained from the animals inoculated with the virulent prophylactic showed a bactericidal value from

about five and one-half to twelve times as great as that obtained from the injection of corresponding amounts of the avirulent. In Table VI the animals of the "virulent" series showed sera from six to fifteen times as great as those of the "avirulent" ones. In Table VII, with subcutaneous inoculation (Nos. 399, 400, 423, and 184), the proportion is from eight to eleven times as great; and in Table VIII, with the dry prophylactic, the value is from one and one-third to four times as great. The results obtained with the dry prophylactic are certainly not so accurate as those with the fluid, on account of the manipulations to which the powder was subjected; and since they are not in accord with all the other numerous experiments, in which the liquid prophylactic was employed, they must be discarded in this comparative consideration. With this exception the results here reported (with the free receptors) are in harmony with those which have been obtained by other observers who for inoculation have employed strains of the killed organisms of different virulence; namely, that the immunity obtained is within certain limits proportional to the virulence of the inoculated strain.

Upon comparing the immunity obtained by the intravenous injection of the prophylactic into rabbits with that produced in the same manner by the inoculation of the living organisms, we see that by the injection of 1 cubic centimeter of the virulent prophylactic (representing the number of receptors obtained from 2 *oesen* after two days' digestion), there is occasionally obtained a serum nearly equaling in bactericidal and agglutinative properties that produced by the intravenous injection of one-half *oesen* of the living virulent organisms. (Compare the animals comprising Table I with those of Table III, particularly animals Nos. 58 and 60.) By a single intravenous injection of 6 cubic centimeters of the prophylactic (obtained from 12 *oesen* after five days' digestion), a serum of far greater value was produced, namely, one agglutinating in dilutions of 1 to 900 to 1 to 1000 (1 milligram) and showing a bactericidal value as high as 1 to 24,000 (0.04 milligram). (See Table V.) Therefore by a judicious use of this method of autolytic digestion a means is offered us of producing by a single intravenous injection into rabbits a serum of greater bactericidal and agglutinative value than could be produced through the employment in the same manner of either the killed or the living organisms. It is known that an agglutinative value of 1 milligram and a bactericidal one of

0.04 milligram are not to be usually obtained by the single injection of cultures of either the killed or the living cholera vibrios, or even when the inoculation is repeated.

EXPERIMENTS ON GUINEA PIGS, SHOWING THE PROTECTION AFFORDED BY THE PROPHYLACTIC, ETC.

From six to twelve guinea pigs were inoculated intraperitoneally and subcutaneously with varying amounts of each of prophylactics I, II, III, and IV and of dried prophylactics II and III, redissolved in normal saline solution. The dose varied from 1 to 5 cubic centimeters. From seven to ten days after the injection of the prophylactic, the animals received intraperitoneally either five or ten times the fatal dose of the living virulent cholera strain. When the virulent prophylactic was employed the animals were invariably protected; but when the avirulent one was used in small amounts of 1 to 2 cubic centimeters, the guinea pigs sometimes succumbed to the subsequent injection of the living organisms. A few of the animals which received large amounts (5 cubic centimeters), of the virulent prophylactic intraperitoneally succumbed, evidently on account of its toxic effects. Upon autopsy no injection of the vessels or hemorrhages at the point of inoculation were observed, such as are always found when death occurs from the inoculation of the living or the dead organisms. Neither were there any hemorrhages in the serous surfaces of the peritoneum. The most noticeable lesions in these cases consisted of a marked edema of the abdominal walls with some flakes of fibrin over the liver. Microscopically it was observed that an extensive desquamation of the epithelial cells had occurred. The contents of the abdominal cavity were sterile.

However, $\frac{1}{2}$ cubic centimeter of the thick material, consisting of the debris of the bacteria which accumulates at the bottom of the flask in the manufacture of the prophylactic, and remains behind on the filter in the form of an emulsion after the prophylactic is passed through, when injected intraperitoneally, causes the death of guinea pigs, in which are found post-mortem the most extensive reaction locally at the point of inoculation and throughout the abdominal cavity, consisting of hemorrhagic areas, injection of the larger vessels, and corrosion of the subcutaneous tissues. These effects evidently are caused by toxic substances forming a constituent of the bacterial membrane not soluble in aqueous solution

after autolytic digestion and which have probably little to do with the production of the true immunity against the disease.

The following experiment shows the comparative value of the protection furnished by the virulent prophylactic and that given by the injection of the living organisms. Four large guinea pigs of about the same weight were chosen. Two were inoculated intraperitoneally with one-fifteenth *ose* of the living virulent organisms and two with 5 cubic centimeters of the virulent prophylactic IV. One of those which received the living organisms was very sick for twenty-four hours following the inoculation. After six days all the animals were reinoculated intraperitoneally with 2 *oesen* of the living virulent strain. The two which had received the prophylactic previously lived; the other two died. Evidently the protection furnished the guinea pigs by the prophylactic was greater than that furnished by the previous injection of one-fifteenth *ose* of the living organisms, a supposition also borne out, and in a more striking way as previously noted, by comparing the immunity obtained by the intravenous injection of the prophylactic into rabbits with that obtained by the injection of the living organisms. From the foregoing it is evident that the virulent prophylactic forms a reliable and *certain* means of protecting guinea pigs against the subsequent injection of multiple fatal doses of the cholera spirillum.

A more extensive study and trial of the bacterial extracts, consisting of the free receptors of the organism in the preparation of prophylactics and sera, would seem to be advantageous. Certain bacterial extracts already have been shown to be of considerable value. Thus, R. Koch, as early as 1891, prepared his original tuberculin by killing, through heating in the steam sterilizer for one hour, four weeks' glycerin bouillon cultures of the tubercle bacillus, evaporating the cultures to one-tenth of their volume, and finally separating the soluble substances from the bacterial cells by filtration. Behring and Landmann, by a somewhat similar method, prepared extracts from the tubercle bacillus, which were said to possess powerful toxic properties. These authors recommended their extracts for the treatment of early cases of tuberculosis, and for the preparation from horses of a curative serum for the disease. Nocard applied a method, somewhat similar to that employed by R. Koch in the preparation of tuberculin, to *Bacillus mallei* for the production of mallein. Still more recently, Conradi, and Neisser

<i>Rabbit</i>			
No.	Intra	000	Control Animals Without Serum.
395	5 c.c. Vir		
396	4 c.c. Vir		
397	1 c.c. Vir		
398	2 c.c. Vir		
420	1 c.c. Vir Preserved		1 control animal; reaction neg; dead in 24 hours.
421	1 c.c. Vir Preserved		1 control animal; reaction neg; dead in 24 hours.
422	2 c.c. Vir Preserved	*	1 control animal; reaction neg; dead in 24 hours.
434	3 c.c. Vir Preserved		
424	1 c.c. Vir reheated		
425	3 c.c. Vir reheated		
438	1 c.c. Vir (in chlo		
439	$\frac{1}{2}$ c.c. Vir (in chlo		

and Shiga,¹ have suggested the use of these extracts in the preparation of a curative serum for typhoid fever and dysentery. However, Conradi advocates the autolytic digestion of the organisms under more natural conditions—i. e., without previous destruction of the bacilli by heat.

We have already begun in the Biological Laboratory experiments relating to the production of a more satisfactory plague prophylactic along somewhat similar lines to those we have employed in the preparation of the cholera prophylactic.

STUDY OF THE TOXIC ACTION OF THE PROPHYLACTIC.

As stated previously, in a few preliminary experiments made with the intravenous injection into rabbits of the virulent prophylactic, if the organisms had been killed by only a very brief period of heating and then allowed to digest themselves, the animals all succumbed to the inoculation. In order to study carefully the agglutinative and bactericidal value of the sera of the inoculated animals, an attempt was made to weaken the toxic action by prolonged heating at 60° C. This heating apparently had the desired effect, as the rabbits then usually survived the inoculations. It then became important to study this toxic action more closely. Accordingly, a prophylactic was prepared by killing the organisms within a very brief period, digesting at 37° C., grinding, submitting them to a pressure of about 600 atmospheres, and finally filtering under pressure through a Reichel or Berkefeld candle. Varying quantities of the prophylactic were then injected intravenously into rabbits. A series of such experiments may be seen in Table IX, and in the case of the control animals of Tables X, XI, and XII.

From these experiments we see that 2 cubic centimeters of virulent prophylactic V prepared after this manner, when injected into rabbits, caused the death of these animals within twenty-four hours. Four and five cubic centimeters injected in the same way produced death in a much shorter time. However, the animals sometimes recovered from the injection of 1 cubic centimeter and were afterwards immune. With virulent prophylactic VI, in which the filtration was performed under pressure with a coarser Berkefeld filter and the bacteria subjected to a more thorough crushing

¹Since the above was written Shiga has advocated the use of the free receptors as a prophylactic against typhoid fever, and Martin Mayer has obtained interesting results from autolysis of bacteria following precipitation with weak ammonium sulphate solutions.

process, even one-half cubic centimeter caused the death of rabbits. In the animals which succumbed to the inoculation, it was found upon post-mortem examination that the kidneys were swollen and showed other evidences of parenchymatous nephritis. The mesenteric vessels were deeply injected and the liver congested and swollen. The lungs showed patches of congestion, hemorrhages, and in one or two cases small pneumonic areas. In a few of the animals there were hemorrhages in the peritoneal surface of the small intestine.

Heating the organism at 60° C. evidently destroys most of the primary poison, or, at any rate, converts the toxine into toxoid, since it is necessary, in order to bring about the death of the guinea pigs, to inject intraperitoneally relatively large amounts (3 to 5 cubic centimeters) of the heated prophylactic. It would seem that the presence of a toxoid would be more desirable in a human prophylactic than that of the unchanged toxine, the toxoid, through the presence of its haptophore group (although its toxophore group is mainly destroyed) still being able to produce antitoxine in the inoculated body without unfolding its general poisonous effect. Such a result we are able to obtain from the injection of our cholera prophylactic, as may be seen from the experiments recorded in Tables X and XI, which are self-explanatory. From Table X it is evident that 3 cubic centimeters and even 2 cubic centimeters of the serum of a rabbit (animal No. 422), which had previously been inoculated with 2 cubic centimeters of the virulent heated prophylactic V, protected other rabbits against two or three times the intravenous dose fatal for these animals; while 2 cubic centimeters of human serum, obtained from a man previously inoculated subcutaneously with 3 cubic centimeters of virulent prophylactic V, when inoculated into rabbits were capable of neutralizing about four times the dose of toxine fatal for these animals. In the experiments shown in Table XI, it may be seen that one-fifth cubic centimeter of the serum of animal No. 423 (previously inoculated subcutaneously with 5 cubic centimeters of virulent prophylactic V) protected a rabbit against about four times the fatal dose of the toxine. In all of these experiments the prophylactic and the serum were mixed immediately before inoculation. The control rabbits without serum died; this being true also in cases in which equal amounts of normal serum were added to the prophylactic before injection. The results recorded in Tables X and XI were the best

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Rabbit No.
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¹ Animal
(See Table IX)
² Human

on.
(V.)

Rabbit No.
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¹ Animal
bleeding one w

by

that could be obtained. When smaller quantities of the serum were employed, the death of the animals always resulted. Indeed, neither would 2 cubic centimeters of the immune serum protect against any higher doses of the toxine. It is admitted that such antitoxic values of the sera, obtained from animals previously inoculated with the prophylactic, are not high; though perhaps better antitoxic properties can be produced when more improved methods are employed to extract the intracellular toxine.

In certain experiments recently performed, in which the digested bacteria before filtration were ground in a mortar with fine quartz sand and infusorial earth, a greater toxic effect on the guinea pigs was observed, these animals dying from intraperitoneal injections of one-half to 1 cubic centimeter of such a fluid. Judging from my own experience in this respect it would appear that the most advantageous method for the extraction of the intracellular toxine of the cholera spirillum would be the one which Macfadyen has recently applied with the same end in view to the typhoid bacillus. By this method the bacteria are ground at the temperature of liquid air, the disintegration having occurred under conditions which precluded the possibility of chemical change. It would seem that a combination of these two methods would perhaps furnish a more ideal prophylactic against Asiatic cholera, namely, the method of autolytic digestion which I have described for obtaining the substances which give rise to the bacterial immunity, and the method of Macfadyen for the extraction of the toxine, the prophylactic consisting of a mixture of the products of both of these procedures carefully heated at such a temperature as to change the larger portion of the toxine into toxoid. In his experiments Macfadyen obtained a toxine from the typhoid bacillus which would kill guinea pigs in intraperitoneal doses of two-tenths cubic centimeter, and which in monkeys gave rise to an immune serum. One-tenth cubic centimeter of this serum protected guinea pigs against a fatal dose of the toxine. My own experiments show that with the modified and extracted toxine no local reaction, similar to that produced by the living or the killed cholera organisms, is obtained, even when sufficient amounts of the former are injected to cause death. The problem, therefore, which confronts us, is the extraction of the toxine in larger quantities. From a few preliminary experiments, already performed with crude apparatus, it would seem that, when the appliances and the methods recommended by

Macfadyen are employed its isolation should be more successful. My experiments throw little light on the nature of the structure of this intracellular toxine. That the haptophore group is identical in structure with that of the soluble toxines of diphtheria and tetanus bacilli would appear doubtful.

The effect of boiling upon the prophylactic.—In Table XII there is recorded a series of experiments¹ showing the effect of boiling upon the toxic action of the prophylactic when injected into rabbits. From these experiments it is seen that the toxic action was destroyed by a temperature of 100° C., since, after the prophylactic had been thoroughly boiled, neither an intravenous injection of 1 cubic centimeter nor one of 2 cubic centimeters caused the death of the rabbits inoculated with it; while control animals receiving 1 cubic centimeter of the unboiled prophylactic always died. Control animals receiving 2 cubic centimeters of peptone solution of the same specific gravity as the prophylactic were unaffected. Animals which received the boiled prophylactic were apparently but little disturbed by the inoculation, and their blood showed practically no agglutinative action with the virulent strain.

Effect produced upon toxic action of prophylactic by its preservation with chemicals.—After several weeks' preservation of the prophylactic in chloretone at room temperature, it was found that a loss of toxic power had resulted, and that evidently there had been a further change of the toxine into toxoid. From the experiments recorded in Table XIII, it is apparent that after preservation of the prophylactic for three months in chloretone neither 1 cubic centimeter nor even 2 cubic centimeters of it, when injected intravenously into rabbits, caused the death of these animals; though the injection of the latter amount produced illness with a rise of temperature of about two degrees. On comparing these results with those obtained by the use of the fresh prophylactic, we see from animal No. 439 in Table IX that formerly $\frac{1}{2}$ cubic centimeter of this prophylactic brought about death, and that 1 or 2 cubic centimeters always produced this effect. (See animals Nos 438, 467, etc.) However, the agglutinable substance of the prophylactic preserved for three months in chloretone remained apparently unchanged in both its groups; since relatively the same amounts of agglu-

¹In connection with the following experiments I wish to express my thanks to Mr. Charles B. Hare, Assistant Bacteriologist in this Laboratory, for much aid.

Rabb No.
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Rabbit No.	Inoculated with	virulent organism only)				
		1:800	1:1,000	1:10,50	1:11,50	1:1200
460	1 c.c. Virulent Prophylactic in chloretone (1 c.c. = 8000)	N	N			N
461	1 c.c. Virulent Prophylactic V in chloretone + 1 c.c. norm Na. Cl. solution.	W	W			N
462	2 c.c. Virulent Prophylactic V in chloretone.	W	N			
509	2 c.c. Virulent Prophylactic V in chloretone.	W	W	N	N	N

*Resuited
with 1/2 chloretone.*

tinine were produced in the sera of the rabbits inoculated with the old prophylactic as were formed in those of the animals inoculated with the fresh one, values as high as 1 milligram being obtained. (See animals Nos. 461 and 509, Table XIII.) The bactericidal values of the sera of the animals comprising Table XIII were not investigated. There was no reason to suppose that any loss in bactericidal power would be found in these sera, since it is well known that the substances giving rise to the bacteriolysins are not so unstable as those which produce the agglutinins.

In regard to the preservation of the prophylactic with 0.5 per cent carbolic acid for a long period of time, practically the same effect as that produced by chlore-tone has been observed, namely, that there is a weakening of the toxic action, so that, where formerly 1 and 2 cubic centimeters of the freshly prepared prophylactic produced death in a rabbit, after a long preservation in 0.5 per cent carbolic acid, 3 cubic centimeters were required to bring about such a result. (See animals Nos. 421, 422, and 434, Table IX.) However, the substances giving rise to the agglutinins and the bacteriolysins are apparently not unfavorably affected by this process. (See animal No. 423, Table VII.)

As already stated, each successive heating of the prophylactic at 60° C. or over, alike unfavorably affects the toxic as well as the agglutinable substances. When the prophylactic is boiled these are apparently destroyed. However, by a careful heating at 60° C. for fifteen minutes the toxic action apparently does not disappear entirely.

On account of the unfavorable effects of the substances mentioned above it has usually been our practice so to handle the prophylactic in its manufacture that it is received from the filter into the sterile tubes, thus making unnecessary any further sterilization either by heat or by the addition of chemicals. With the prophylactic preserved in a sterile manner and kept at the temperature of the ice box, I have obtained very good results five months after its preparation. Of course, the toxic action becomes weaker after a short period of time, and this process gradually increases, owing to the still further change of toxine into toxoid.

HUMAN INOCULATIONS.

After studying the effects of the prophylactic upon animals, it was also desirable to ascertain its action upon human beings. With

this end in view, a number of individuals have been inoculated from time to time with varying amounts (1 to 5 cubic centimeters) of the virulent prophylactic. The inoculations have been made deep into the muscles of the arm. In these cases the local reaction was never very marked. There was usually soreness on pressure in the region of the inoculation, lasting for about twenty-four hours, and occasionally a slight reddening of the overlying skin was observed. None of the patients have complained of much pain. No suppuration has ever been observed, and in fact it may be said that the local reaction is very slight. Following the inoculation there was generally a rise of temperature of from 1° to 3° (Fahrenheit), which subsided in from twenty-four to forty-eight hours. Headache, lasting for a few hours, was occasionally complained of. Unfortunately for a further trial of the method, there has not been sufficient cholera present in the city or in the provinces during the past nine months to warrant the introduction of a general inoculation of the people against this disease; nor has there been any opportunity to observe the immunity of the inoculated from an entirely practical standpoint. The fact, therefore, that no cases of cholera have occurred among those receiving the prophylactic shows nothing in regard to the value of the method, since it is doubtful to what extent they have been exposed to the disease.¹ However, it has been demonstrated that the blood sera of the inoculated individuals, both white and native, acquire protective substances. The results of a study of a number of these cases may be seen in Table XIV; before the injection the serum of none of them showed any agglutination of the virulent organism in dilutions of 1 to 20 (50 milligrams), or any bactericidal action in dilutions of 1 to 50 (20 milligrams). The blood was drawn from one of the veins of the arm one week after the inoculation, and after the separation of the serum the value of the latter was determined. From these experiments it appears that 3 or 4 cubic centimeters of the prophylactic furnished the best sera, namely, those having an agglutinative value against the virulent strain of from 4 to 2.5 milligrams and a bactericidal one of from 0.33 to 0.25 milligram. These sera are much more potent than those obtained in human beings by Kolle from the subcutaneous injection

¹ Attempts at subsequent infection of the inoculated by feeding living cultures of cholera spirilla have shown themselves so unsatisfactory in the past that for this reason and other obvious ones they have not been resorted to.

<i>Case</i>		<i>Inoculum</i>
<i>No.</i>	<i>Subcutaneous</i>	<i>Control Animals Without Serum.</i>
1	3 C.C. V. Prophyl. 1 C.C. -	2 control animals; reactions neg.; all dead in 24 hours.
2	3 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
3	2 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
4	2 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
5	2 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
6	2 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
7	1 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
8	1 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
9	1 C.C. V. Prophyl. 1 C.C. -	1 control animal; reaction neg.; dead in 24 hours.
10	4 C.C. V. Prophyl. 1 C.C. -	2 control animals; reactions neg.; all dead in 24 hours.

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of either the living or the killed cholera spirilla. Kolle's best sera showed a bactericidal value of from 3 to 1.5 milligrams. They also show a higher value than is usually seen in the sera of human beings who have recovered from an attack of Asiatic cholera, which, according to the investigations of R. Pfeiffer and of Kolle, may be 10 milligrams. Therefore, we might presume that a good active immunity had been acquired against the disease by the use of this prophylactic. The antitoxic value of the sera has already been discussed. In case Number 1, two cubic centimeters of the serum protected rabbits against four times the intravenous dose, fatal for these animals.

We have seen that by the subcutaneous injection of the cholera prophylactic an excellent cholera immune serum can be obtained in human beings. However, the question naturally arises, whether these individuals are protected against intestinal infection with the cholera spirillum. In other words, are they really immune to the disease Asiatic cholera? Experiments upon animals can not satisfactorily answer this query. The earlier investigations of Brieger, Kitasato, Wassermann, Haffkine, and others upon the point at issue, namely, whether animals could be rendered immune against intestinal infection with Asiatic cholera, spoke in the affirmative. However, the more recent work of Pfeiffer, Wassermann, and Sobernheim demonstrated that immunity in animals against such infection was not certainly to be obtained by the ordinary methods of immunization then in vogue. Since animals are not naturally susceptible to intestinal infection, and since it is only through artificial means that such may be produced in them, evidently the answer to our question can be given only by a practical observation of the human beings inoculated with the prophylactic during a severe and general epidemic of the disease. For this reason it was hoped that a more extensive practical demonstration of the value of the prophylactic could be given before an extended publication of the work was made.¹

However, since the present report has been delayed nearly nine months, and as it appears that there will be no greater opportunity in the near future for a more practical test of the prophylactic in these Islands than has already been experienced, it is thought inadvisable to defer for a longer period the publication of the

¹The results of the experimental work were presented to the Manila Medical Society at the meeting of September 7, 1903.

experimental work. Moreover, it would appear, from the numerous statistics of Haffkine in India, and the more recent work of Murata in Japan, that simply by the injection of a small amount of the killed organisms a certain degree of immunity against the natural mode of infection is acquired. Therefore, judging from what has already been said, it is probable that by the use of our prophylactic, human beings may acquire a good active immunity against the disease.

CONCLUSIONS.

(1) By the autolytic digestion of carefully killed cholera spirilla in an aqueous fluid the receptors become separated from the bacterial cells and may be filtered off in solution.

(2) The injection of these free receptors into both man and animals furnishes a means of producing high bactericidal and agglutinative blood sera. The antitoxic value of these sera is, however, moderate.

(3) The subcutaneous injection into man of such free receptors is a process which is not only free from any danger but one which produces practically no local disturbance and only a slight general reaction.

(4) Hence the method is a practicable one for producing a cholera immune serum in man.

(5) It is highly desirable that this cholera prophylactic be given a thorough, practical test.

(6) It would appear hopeful that by the application to the pest bacillus of a slight modification of this method a more satisfactory prophylactic against bubonic plague could be obtained.

Experiments with this end in view have already been commenced in the Biological Laboratory.

The consideration of the comparative results in immunity obtained with the inoculation of the virulent and the avirulent living organisms and with the prophylactics of different virulence will be considered in another article.

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1904.—No. 17.

DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

NEW OR NOTEWORTHY PHILIPPINE PLANTS, II.

BY ELMER D. MERRILL, BOTANIST.

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LETTER OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., June 15, 1904.

SIR: I have the honor to transmit herewith a paper entitled
"New or Noteworthy Philippine Plants, II," by Elmer D. Merrill,
Botanist.

I am, very respectfully,

PAUL C. FREER,
Superintendent of Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior.

LIST OF ILLUSTRATIONS.

- PLATE I. *Pandanus luzonensis* Merrill, habit.
II. *Pandanus luzonensis* Merrill, fruit.
III. *Pandanus arayatensis* Merrill, fruit.

NEW OR NOTEWORTHY PHILIPPINE PLANTS, II.

By ELMER D. MERRILL.

The descriptions of the new species proposed in the present paper, and the notes on those previously described which are of interest either on account of their distribution or nomenclature, have been prepared from time to time as material and data became available. The types of the new species proposed are deposited in the herbarium of the Bureau of Government Laboratories, the present paper being based entirely on material contained therein. The first paper of this series was issued as Publication 6 of the Bureau.

TAXACEÆ.

PODOCARPUS BLUMEI Endl. Syn. 208. 1847; Pilger in Engler, Pflanzenreich, 18:60. 1903.

This widely distributed species, not previously known from the Philippines, is abundant on Mount Mariveles, Province of Bataan, Luzon, growing on forested slopes and ridges at and above an elevation of 700 m. above the sea, associated especially with *Agathis philippinensis* Warburg. No. 147 Forestry Bureau, collected by Barnes, January, 1904; No. 244, Cope-land, January, 1904, both specimens with mature fruit.

PANDANACEÆ.

FREYENETIA ENSIFOLIA Merrill, sp. nov.

A scandent, sparingly branched shrub 2 to 3 m. high, with narrow, linear-lanceolate leaves, globose fruits and ovate angular drupes which have from three to six, generally five, stigmas. Branches triangular, glabrous, reddish-brown, 4 mm. thick, the internodes 1 cm. long below, 6 mm. long above. Leaves 7 to 11 cm. long, 5 to 7 mm. wide, gradually narrowed to the slender, scarcely acuminate apex, the base rather abruptly narrowed at a point about 1 cm. from its insertion, scarcely sheathing, glabrous, the margins entire, except at the scabrous apex and near the base of the

leaf where they are finely serrate. Inflorescence terminal, the pistillate spadices in threes, globose after flowering, 1 to 1.5 cm. in diameter; peduncles glabrous 1 cm. long. Drupes ovate, angular, 5 mm. long, 3 mm. in diameter at the middle, the base narrowed, the apex contracted, flattened, about 1 mm. in diameter. Stigmas 3 to 7, usually five. Seeds linear-lanceolate, 2.5 mm. long.

Type specimen No. 3242, Merrill, Mount Mariveles, Province of Bataan, Luzon, October, 1903; growing on exposed wind-swept ridges at an elevation of about 1,200 m.

A species of the section *Pleio stigma*, evidently related to *Freydenetia sphaerocephala* Gaud., but abundantly distinct.

PANDANUS LUZONENSIS Merrill, sp. nov. § *Vinsonia*. Plates I, II.

An arborescent species about 8 m. high, with very long, narrow leaves and solitary, globose, pendant fruits, 8 to 10 cm. in diameter. Trunk erect, 10 to 12 cm. in diameter, much branched above, the branches spreading or ascending, the ultimate ones 2 cm. in diameter. Leaves 1.5 to 2 m. long, 2.5 to 3 cm. wide, glabrous, the apex very long narrowly acuminate, the margins strongly antrorsely toothed throughout, the midrib beneath, below with stout retrorse, curved spines, smooth in the middle, and above with small antrorse spines, the upper surface glabrous except near the apex where there are two rows of small, scattered teeth between the margins and the midrib, the margins at the apex finely serrate. Staminate inflorescence 20 to 30 cm. long, fleshy, branched, the branches thick, ascending, the lower ones 8 cm. long, each branch subtended by a broad, thin bract, 8 cm. wide, the lower one abruptly contracted and the tip foliaceous, 30 cm. long, or longer, the second and third bracts abruptly acuminate, the tips more or less foliaceous, the upper bracts much smaller, acute. Stamens 4 to 9, the filaments united into a fleshy tube 4 to 8 mm. long; anthers 2 mm. long. Pistillate inflorescence unknown. Fruit solitary, the peduncle triangular, gradually thickening upward, 1 cm. thick above, 20 cm. long. Drupes 30 to 60 in each head, yellowish red when ripe, ovate or obovate, 3 to 4 cm. long, 2 to 2.5 cm. thick, smooth and shining, sharply 5 angled, the upper third free, convexly pyramidal, the apex flattened, 5 to 10 mm. in diameter, slightly sulcate, the stigmas flattened, often obscure. Seeds 6 to 10.

Type specimen No. 3317, Merrill, Lamao River, Province of Bataan, Luzon, October, 1903, altitude 100 m. (fruit); No. 92 Forestry Bureau; collected by Barnes, same locality November, 1903 (staminate inflorescence); Subig, Province of Zambales, Luzon, H. Hallier, December, 1903.

This species is very common along the Lamao River and on forested slopes of Mount Mariveles up to an elevation of about 600 m. above the sea, being known to the Tagalogs of Bataan as *Pandan*, a name generally applied by them to all species of *Pandanus*, and some species of *Freydenetia*. It is apparently most closely related to *Pandanus sylvestris* Bory, from the Island of Reunion, differing in its larger size, longer leaves, larger drupes, etc.



PLATE I. *PANDANUS LUZONENSIS* MERRILL, HABIT.

PANDANUS ARAYATENSIS Merrill, sp. nov. § *Keura*. Plate III.

A stout, erect, branched shrub about 4 m. high, with numerous aerial roots from the lower part of the stem, the leaves crowded at the ends of the branches, about 2 m. long, 6 to 7 cm. wide, and solitary, ellipsoid, pendant, glaucous fruits 14 to 16 cm. long. Trunk erect, 10 to 13 cm. in diameter, with very small white, scattered, pyramidal spines, the few branches from the summit spreading, 4 to 5 cm. in diameter, aerial roots numerous, 3 to 4 cm. in diameter, ascending to a height of about 1 m. on the trunk. Leaves about 2 m. long, 6 to 7 cm. wide, the apex abruptly acute, not acuminate, dark green, glabrous, except for the toothed midrib and margins, shining above, dull beneath, the midrib glabrous above, beneath also glabrous, except near the apex of the leaf where it is more or less antrorsely toothed, margins strongly antrorsely toothed throughout. Fruit green, glaucous, becoming yellowish when ripe, ellipsoid, 15 to 16 cm. long, 12 to 13 cm. thick, the peduncle 3 dm. long, triangular, thickened above, the fruit consisting of from 110 to 120 closely packed, very hard drupes, less than 1 cm. of the drupes free at their apices. Drupes 4.5 to 5 cm. long, about 1 cm. thick at the base, 2 to 3 cm. thick at the apex, irregularly 4 to 6 angled, the apex flattened, sulcate; locules 10 to 13, irregularly disposed, their apices conical or pyramidal, often irregularly so, the separating sulci one-third to one-half as deep as the sulci separating the drupes; stigmas 1 to 2 mm. in diameter, more or less oblique.

Type specimen No. 3832, Merrill, Mount Arayat, Province of Pampanga, Luzon, May 15, 1904; a species found in the forests only at and near the summit of the mountain at an altitude of 878 m.

PANDANUS COPELANDI Merrill, sp. nov. § *Rykia*.

An erect, unbranched plant about 2 m. high. Leaves 1 to 1.5 m. long, 5.5 to 6 cm. wide, glabrous, shining above, glaucous beneath, the margins and the midrib beneath spinously toothed, the teeth pale, coarse below, 3 to 4 mm. long, the marginal teeth antrorse throughout, those on the midrib retrorse below, antrorse above, at a point 6 or 7 cm. from the apex of the leaf the leaf is 3.5 cm. wide, and from this point it tapers abruptly to the acute, not acuminate, apex, the midnerve and margins of the apex finely and densely serrate. Flowers unknown. Rachis triangular, 1.5 cm. thick, 50 cm. long. Fruits solitary or racemed, nearly sessile, elliptical or subspherical, 7 to 9 cm. long, 5 to 6 cm. in diameter, red when mature. Drupes very numerous, 14 mm. long, 3 to 4 mm. in diameter, the apex conical, 10 mm. long, the slender style proper, 5 to 6 mm. long, entire, curved upward. Endocarp 8 mm. long.

Type specimen No. 140, Copeland, Gimagon River, Negros, January 5, 1904; growing in forests at an elevation of about 100 m.

A species of the section *Rykia*, evidently most closely related to *Pandanus sarasinorum* Warb., a Celebes species, differing from the latter in its wider leaves, smaller fruits, thicker drupes, and longer style.

PANDANUS WHITFORDII Merrill, sp. nov. § *Sussea*.

A small, erect, branched shrub, 1.5 m. high or less, with narrow, linear leaves, about 1 cm. wide, and small globose fruits, 1.5 cm. in diameter or

less, which are crowded in a dense erect raceme. Trunk erect, 3 to 5 cm. in diameter, branched from the top, the branches spreading, 1 cm. in diameter. Leaves 50 to 60 cm. long, 10 to 12 mm. wide, tapering above to the long, narrowly acuminate apex, below abruptly widened into a clasping base, which is 2.5 cm. wide, the margins throughout antrorsely scabrous, the midrib glabrous on the upper surface, beneath antrorsely scabrous in the upper portion of the leaf, retrorsely scabrous in the lower portion, some of the nerves antrorsely scabrous near the apex. Peduncle slender, 15 to 20 cm. long, the fruit crowded at the apex, three or four fruits in a raceme, each subtended by a broad bract, the first bract 8 cm. long, 1.5 cm. wide, very long acuminate, the upper bracts gradually shorter but nearly as wide, very abruptly acuminate. Fruits globose, each subtended by a broad-based bract, sessile, 1.5 cm. in diameter or less. Drupes, about 50 in each fruit, 1 celled, obovoid, yellow when mature, 7 mm. long, 4 mm. thick or less, the apex rounded, rarely slightly depressed, the stigma in the center, 1 mm. thick or less.

Type specimen No. 351, H. N. Whitford, Mount Mariveles, Province of Bataan, Luzon, May 31, 1904.

A very common species in this locality, growing as an undershrub on forested slopes and ridges at an elevation of from 600 to 1,100 m. above the sea. Rarely found in fruit.

PANDANUS DUBIUS Spreng. (*Pandanus radicans* Blanco.) § *Hombromia*.

This species has previously been known from New Guinea, the Moluccas, and Polynesia, as far north as Guam, and has recently been collected in the Southern Philippines by E. B. Copeland, No. 613, sea coast, below Malalag, district of Davao, Mindanao, March, 1904. *Pandanus radicans* Blanco is undoubtedly a synonym of this species.

PANDANUS POLYCEPHALUS Lam. § *Sussea*.

This species, which previously has not been definitely known from the Philippines, extends from the Malayan Archipelago to New Guinea. Nos. 386 and 442, E. B. Copeland, Davao, Mindanao, March, 1904. A species generally found in the *Barringtonia* formation along the sea coast.

GRAMINEÆ.

PANICUM NITENS Merrill, sp. nov. § *Effusa*.

A lax, branched, glabrous suberect perennial grass 30 to 90 cm. high, with thin, lanceolate, acuminate leaves, lax, few-flowered panicles, and lanceolate, glabrous spikelets 4 to 4.5 mm. long. Culms slender, not tufted, glabrous and shining, usually purplish, rooting at the lower nodes, suberect, much branched; nodes glabrous or nearly so. Sheaths lax, 2 cm. long, much shorter than the internodes, the margins clothed with long, soft, white hairs; ligule a very short ciliate ring. Leaves 8 to 10 cm. long, 8 to 12 cm. wide, thin, glabrous, shining, tapering to the slender apex, the base rather abruptly contracted, the margins glabrous or slightly scabrous. Panicles terminal and axillary, usually contracted, the rachis and branches glabrous or slightly scabrous, terminal panicles much exserted, about 10 cm. long, the branches 5 to 7 cm. long, few flowered, the axillary panicles from the upper sheaths slender, much

elongated. Spikelets lanceolate, acuminate, 4 to 4.5 mm. long, solitary, equaling or usually shorter than their scabrous pedicels; empty glumes lanceolate, acuminate, subequal in length, more or less scabrous on their nerves, otherwise glabrous; first glume thin, white, with three prominent nerves, narrower than the other two, 3 to 3.5 mm. long; second glume 4 mm. long, 5 nerved; third glume 4 mm. long, 5 nerved, with a thin 2-nerved palea 2.5 to 3 mm. long. Flowering glume coriaceous, elliptical-ovate, obtuse, smooth and shining, 2 to 2.2 mm. long, 1 mm. wide. Palea equaling the glume.

Type specimen No. 3756, Merrill, exposed ridges, Mount Mariveles, Province of Bataan, Luzon, January 1, 1904, growing on ledges at an elevation of 1,200 m. above the sea; No. 3221, Merrill, same locality, October, 1903, is a small form of this species.

OPLISMENUS MINUS Merrill, sp. nov.

A low, decumbent grass, with small, lanceolate leaves and solitary or clustered spikelets, forming a simple terminal spike. Culms slender, 2 dm. long or less, branching and rooting at the nodes, the flowering branches suberect; nodes with few ciliate hairs. Leaves 1.5 to 3 cm. long, 3 to 5 mm. wide, thin, glabrous, or with few long, white hairs, the base abruptly contracted, the apex acute or acuminate; sheaths 0.5 to 1 cm. long, equaling the internodes. Spikes simple, erect, 3 to 5 cm. long. Spikelets 1 to 3 at each node, 3 mm. long, excluding the awns; empty glumes clothed with few long, white hairs; first glume 3 nerved, 2 mm. long, nearly 1 mm. wide, its awn 6 to 8 mm. long; second glume 5 nerved 2.2 mm. long, 1.2 mm. wide, its awn 2 to 3 mm. long, third glume 7 nerved 2.2 mm. long, 1.5 mm. wide, its awn 1 mm. long. Flowering glume lanceolate, acute 2.5 mm. long, 0.8 mm. wide, smooth and shining, enclosing its palea.

Type specimen No. 3203, Merrill, Mount Mariveles, Province of Bataan, Luzon, October, 1903, growing on exposed ridges at an elevation of 1,300 m.

A species most closely related to *Oplismenus undulatifolius* Beauv., but is in every way much smaller than that species.

CYNODON ARCUATUS Presl, Rel. Haenk. 1:290. 1830; Scribn. Rept. Mo. Bot. Gard. 10:41. pl. 40. 1899.

This species is evidently distinct from *Cynodon dactylon* (L.) Pers., although it is closely related to that species. *Cynodon arcuatus* is distinguished from *C. dactylon* especially in its erect, not prostrate habit, larger size, more numerous and longer spikes, and larger leaves. The spikelets of the two species are practically identical, but in habit and appearance the two species are very different. *Cynodon arcuatus* Presl, is represented in the herbarium by No. 3619, Merrill, Concepcion, Province of Tarlac, Luzon, October, 1903; and No. 3171, Merrill, Lamao River, Province of Bataan, Luzon, October, 1903. A species growing in open, dry grass lands.

CASUARINACEÆ.

CASUARINA NODIFLORA Forst.

This species is represented by No. 1234, Copeland, Todaya, district of Davao, Mindanao, altitude 1,200 m. above the sea, a tree growing in

clearings, ravines, etc., reaching a height of 30 m., known to the people of the Bogobo tribe as *Gó-o*. This species has previously not been reported from the Philippines, extending from the Moluccas to New Guinea, New Caledonia, and the Fiji Islands.

MORACEÆ.

ARTOCARPUS XANTHOCARPA Merrill, sp. nov.

A tree 30 m. high or less, with narrowly ovate or ovate-oblong, acuminate, entirely glabrous leaves, and subglobose more or less irregular, entirely glabrous orange-yellow fruit, 3 cm. in diameter or less. Branches slender, glabrous, light gray. Leaves 8 to 12 cm. long, 3 to 4.5 cm. wide, subcoriaceous, shining, the base slightly rounded or subacute, inequilateral; nerves rather prominent beneath, 8 to 9 pairs, the reticulations lax; petioles glabrous, 1 to 2 cm. long. Flowers unknown. Female receptacles axillary, the individual apocarps entirely united, the surface of the syncarpium very smooth, when dry somewhat sulcate between the apocarps; seeds obovoid, more or less irregularly compressed, 1 cm. long, 4 to 15 in each receptacle. Peduncle of the receptacle 1 cm. long or less.

Type specimen No. 367, H. N. Whitford, Lamao River, Province of Bataan, Luzon, June 7, 1904.

A tree on the wooded slopes at an altitude of 200 m. above the sea, reaching a diameter of about 35 cm., the trunk clear to a height of about 17 m. Bark brittle, the outer bark consisting of thin, reddish, papery scales, exuding a white latex when cut. The Tagalogs of Bataan know this species as *Sulipa*.

Possibly related to *Artocarpus lanceolata* Tréc., a species known only from the Island of Luzon, but that species has more or less pubescent branches and leaves and single-seeded receptacles.

FICUS *NOTA* (Blanco) (*Ficus aspera* var. *nota* Blanco, Fl. Filip. ed. 1, 677. 1837; *Ficus scabra* Blanco, l. c., ed. 2, 471; ed. 3, 3:381, non Forst.; *Ficus racemifera* F.-Vill. Nov. App. 201, non Roxb.) § *Covellia*.

A medium-sized tree, with broadly ovate, cordate, acuminate leaves which are more or less pubescent, and subglobose, green or purplish receptacles which are borne in masses on specialized leafless branches from the trunk and larger branches. Ultimate branches brownish, usually densely pubescent, rarely nearly smooth. Leaves 15 to 25 cm. long, 9 to 15 cm. wide, the margins entire or usually more or less coarsely serrate above, the base cordate, often inequilateral, the apex abruptly short-acuminate, the upper surface usually rather harsh, densely pubescent on the midrib and nerves, and with few scattered hairs on the leaf surface, becoming glabrous or nearly so, the lower surface light brown when dry, usually uniformly softly pubescent throughout with short scattered hairs, often also with numerous small white papillæ; nerves from the base, seven, the outer two obscure, submarginal, main nerves 8 or 9 pairs, ascending, prominent, especially beneath, the nerves and the larger branches anastomosing near the margin, forming a much-arched marginal nerve; petioles 1.5 to 3.5 cm. long, 4 mm. in diameter, densely pubescent; stipules deciduous, ovate-lanceolate, acu-

minate, 1 to 1.5 cm. long. Inflorescence on stout much-branched specialized branches from the trunk and the larger limbs, forming subspherical masses sometimes 5 to 10 cm. in diameter, but often 30 to 60 cm. in diameter or more. Receptacles very numerous subglobose, or slightly pear shaped, about 3 cm. in diameter, green or purplish, with scattered white and reddish spots, slightly pubescent with short spreading hairs, becoming nearly glabrous, the apex flattened or subtruncate, the base rounded or subacute; umbilicus not prominent; peduncle 1 to 1.5 cm. long, 4 mm. in diameter, pubescent; basal bracts at the apex of the peduncle triangular, acute, 4 mm. long. Fertile female flowers sessile or pedicellate, numerous, when pedicellate the pedicels often 4 mm. long; achene 2 mm. long, reddish, style 0.5 mm. long; perianth very small, hyaline, less than 0.5 mm. long. Gall flowers in good condition, and male flowers not seen.

Specimens examined: Luzon, Manila, No. 350, Merrill, July, 1902; No. 3465, Merrill, November, 1903. Province of Rizal, Malapadnabato, No. 2731 Merrill, June, 1903. Province of Bataan, Lamao, No. 2492 Merrill, June, 1903; No. 324, Forestry Bureau, collected by P. T. Barnes, February, 1904; Mariveles, No. 777, Ahern, January, 1902; Balanga, No. 305, Ahern, 1901 (erroneously distributed as from Mindanao.) Province of Zambales, Boto-lan, No. 2902, Merrill, May, 1903. Mindoro, Pola, No. 2471 Merrill, June, 1903. Island of Culion, No. 688 Merrill, February, 1903.

Ficus nota is a common tree in the Philippine forests, both in the low-lands and in the hills, reaching a height of from 8 to 10 m. and a diameter of 25 cm. or less. The abundant, milky sap when coagulated is similar in appearance and physical characters to the gum of *Achras sapota*, "Gum chicle" of commerce, which is so extensively used for the manufacture of chewing gum. This species is well known to the natives, although the native names are also sometimes applied to other species of the genus that have a similar cauline inflorescence. T., *Tibig*; I., *Tecbec*.

FICUS MINAHASSÆ Miq. Ann. Lugd. Bat. 3:231, 296. 1867; King, Ann. Bot. Gard. Calcutta, 1:108. pl. 140, 141. 1888; *Ficus glomerata* Blanco, Fl. Filip. ed. 1, 683; ed. 2, 475; ed. 3, 3:87, non Roxb. § *Covellia*.

This very striking and characteristic species is known only from the Philippines and Celebes, and was first described by Blanco, his name, *Ficus glomerata*, being, however, previously taken by that of Roxburgh's species. *Ficus glomerata* Blanco is certainly a synonym of *F. minahassæ* Miq., as Blanco's description agrees perfectly with the characters of *Ficus minahassæ*. F.-Villar¹ erroneously considered that Blanco described the species of Roxburgh, and accordingly reduced *Ficus glomerata* Blanco to *Ficus glomerata* Roxb., but the only point of resemblance in the two species is in the names. *Ficus minahassæ* Miq. was reported from the Philippines by the author in the year 1903² as a species new to the Archipelago, but at that time the identity of Blanco's *Ficus glomerata* was not established. This species is apparently common and widely distributed in the Philippines, growing especially on river banks in the forests, and is represented by the following specimens: Luzon, Province of Bataan, Lamao River, No.

¹ Nov. App. 201.

² Forestry Bureau, Bull. 1:18.

2534, Merrill, June, 1903; No. 66, Forestry Bureau, collected by Barnes November, 1903, same locality; Province of Tayabas, Guinayangan, No. 2033, Merrill, April, 1903. Mindoro, Baco River, No. 1797, Merrill, April, 1903. Mindanao, Surigao, No. 494, Ahern, 1901; Zamboanga, No. 682, Ahern, 1901. Island of Basilan, No. 26, DeVore and Hoover, April, 1903. T. V., *Aimit*, *Haguimit*, *Ayumit*; V., *Tambis tambis*; T., *Tibig na lalaqui*; (Moro?), *Matanug*.

FICUS BARNESII Merrill, sp. nov. § *Covellia*.

A small tree about 6 m. high, with narrowly ovate, acuminate, membranaceous, rather harsh, densely papillate leaves, the receptacles on long, drooping, slender, slightly branched, specialized, leafless branches from the trunk and larger branches. Young branches rusty brown, rather densely hirsute with reddish hairs. Leaves 13 to 20 cm. long, 5 to 8 cm. wide, narrowed to the acute base which is slightly inequilateral, tapering from slightly above the middle to the slender acuminate apex, the margins entire, both surfaces rather harsh, and densely punctate with small, white papillæ, the upper surface, especially the midrib and nerves, with few scattered white hairs, the midrib and nerves beneath hirsute with brownish hairs; nerves about 9 pairs, ascending, prominent beneath, rather obscure above, anastomosing near the margin, the reticulations rather prominent; petioles rusty brown, hirsute, 1 to 2.5 cm. long; stipules lanceolate acuminate, hirsute, 5 mm. long. Inflorescence about 1 m. long, the branches glabrous, brownish gray. Receptacles light green, glabrous, solitary or two or three from the same branchlet, pear shaped, 2 to 2.5 cm. long, the apex rounded or truncate, narrowed slightly to the rounded base; umbilicus 5 mm. in diameter; peduncle glabrous, 2 cm. long, the basal bracts triangular, 2 mm. long, at the summit of the peduncle. Fertile female flowers sessile or pediceled, the pedicels often 2 mm. long; perianth wanting; ovary red, ovoid-globose, smooth, 1 mm. long; style lateral, 1.5 to 2 mm. long.

Type specimen No. 325 Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, February, 1904. A tree growing in the hill forests on the river bank at an altitude of 200 m.

FICUS MINDOROENSIS Merrill, sp. nov. § *Covellia*.

A small tree 10 to 15 m. high, with light-gray glabrous branches, ovate, acuminate leaves, the receptacles borne on long, pendant, specialized, leafless branches from the trunk and larger branches. Branches smooth, the ultimate branchlets appressed pubescent. Leaves thin, membranaceous, 10 to 15 cm. long, 5 to 8 cm. wide, the margins entire, the base acute or somewhat rounded, the apex acuminate, the nerves and midrib beneath pubescent with appressed white hairs, and both surfaces, but especially beneath, uniformly pubescent, but not papillate, with scattered hairs, the upper surface becoming glabrous; nerves, 5 pairs, ascending, curved, rather prominent beneath, the reticulations lax; petioles appressed-pubescent, 1 cm. long or less. Inflorescence on long pendant more or less branched branches, which are from 0.5 to 1.5 m. long, light gray, glabrous. Receptacles light green, glabrous, pear shaped, 1.5 cm. long, and nearly as wide in the upper part, with scattered wart-like growths, the apex

rounded or truncate, the base subacute; umbilicus prominent, about 4 mm. in diameter; peduncle 6 to 8 mm. long, pubescent, the basal bracts at the apex of the peduncle triangular, not well developed, pubescent. Male flowers few, only near the ostiole, 3 mm. long, the perianth brown, inflated, of three much-imbricated parts, 2.5 mm. long, the solitary anther just exerted from the perianth, white, broadly ovate, 0.8 mm. long, 0.5 mm. wide. Gall flowers sessile or pedicelated, the pedicels often 3 mm. long, perianth wanting, the ovary ovoid-globose, smooth; style 0.4 mm. long, lateral. Fertile female flowers not seen.

Type specimen No. 1813 Merrill, humid forests, Baco River, Mindoro, April, 1903. A species evidently related to *Ficus conora* King, but very distinct; No. 1797, Merrill, which was issued under this name is *Ficus minahassæ* Miq.

FICUS RUFAULIS Merrill, sp. nov. § *Eusyce*.

A small tree, reaching a height of 15 m. with broadly ovate, cordate, acuminate leaves and pedunculate, densely pubescent, axillary, ovoid receptacles, 2 to 2.5 cm. long. Ultimate branches thickened, glabrous or nearly so, reddish brown, the leaf scars large and prominent. Leaves 20 to 25 cm. long, 10 to 15 cm. wide, membranaceous, the margins entire, rounded to the cordate base, tapering above to the usually slender acuminate apex, the upper surface glabrous or somewhat pubescent on the midrib and nerves, the under surface uniformly softly pubescent, with rather scattered white or brownish hairs; nerves prominent, ascending, 6 to 7 pairs, the reticulations lax; petioles brownish red, pubescent, 3 to 7 cm. long. Receptacles ovoid solitary or in pairs from the axils of the leaves or from above the leaf scars, densely pubescent with grayish hairs, the umbilicus prominent, the protecting scales more or less exerted; peduncle densely pubescent, 1 cm. long, with three triangular, pubescent basal bracts at or near the base of the receptacle. Fertile female flowers sessile or pedicelated, when pedicelated the pedicel often 3 mm. long, glabrous or with few appressed white hairs, the base of the pedicel also surrounded by straight white hairs; perianth brownish, four parted, surrounding the achene, 2.5 mm. long, the apices of the segments slightly ciliate; achene subglobose 1.5 mm. long, rugose, the style subterminal, slender, 1 mm. long. Male flowers not seen.

Type specimen No. 1739, Merrill, Antipolo, Province of Rizal, Luzon, March, 1903; No. 2471 Merrill, Pola, Mindoro, June, 1903; No. 512, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, February, 1904, is an immature specimen of the same, while closely related forms are represented by No. 658, Merrill, Island of Culion, February, 1903, but with sessile or nearly sessile receptacles, and No. 698, Ahern, Surigao, Mindanao, 1901, but with very large leaves 30 cm. long, 20 cm. wide, the pedicels of the female flowers strongly pubescent.

In gross characters this species strongly resembles *Ficus fulva* Reinw., but is not at all closely related to that species.

FICUS VARIEGATA Blume, Bijdr. 459; King, Ann. Bot. Gard. Calcutta, 1:189, pl. 212. 1888. (*Ficus lavigata* Blanco, Fl. Filip., ed. 1, 682. 1837; ed. 2, 474; ed. 3, 3:86; *Ficus cuneata* F.-Vill., Nov. App. 202. 1883, non Miq.) § *Neomorphe*.

Ficus lavigata Blanco, was erroneously referred by Villar to *Ficus cuneata* Miq., but is certainly identical with *Ficus variegata* Blume. F. Villar reduced to *Ficus racemifera* Roxb., which is a synonym of *Ficus variegata* Blume, *Ficus aspera nota* Blanco, which is a very distinct species of the section *Covellia*, which shows that he had no clear conception of the species, to which he reduced the species of Blanco, although his conception of Blanco's species may have been correct. *Ficus variegata* Blume is apparently common and widely distributed in the Philippines and is universally known to the Tagalogs by the peculiar name *Tangisang bayauac*, which is explained by Blanco. It is represented in the herbarium by the following specimens, all received under the common name given above. Luzon: Province of Zambales, Botolan, No. 2961, Merrill, May, 1903; Cabangan, No. 3008, Merrill, July, 1903; Province of Bataan, Lamao River, No. 325, Forestry Bureau, collected by Barnes, March, 1904; No. 601, Forestry Bureau, collected by Barnes, same locality, January, 1904; Province of Tayabas, Guinayangan, No. 2022, Merrill, April, 1903.

FICUS MEGACARPA Merrill, sp. nov. § *Synoecia*.

A glabrous vine, ultimately reaching a height of 20 m. or more, and a diameter of 5 or more cm., but when young a slender, profusely branching vine creeping on the trunks of trees. Ultimate branches slender, reddish brown, the tips clothed with few appressed reddish hairs. Leaves ovate or elliptical-ovate, the apex rounded, often slightly emarginate, the base rounded or acute, usually somewhat inequilateral, glabrous, coriaceous, 2 to 5 cm. long, 1.5 to 3 cm. wide, the upper surface grayish when dry, hardly shining, the lower surface with numerous pustules; nerves three or four pairs, not prominent, nearly obsolete above; petioles 3 to 5 mm. long; stipules deciduous, lanceolate, acuminate, 5 mm. long. Receptacles very large, ellipsoid or subglobose, or sometimes somewhat pear shaped, the apex rounded or truncate, the base rounded or subacute, 7 cm. long, 6 cm. wide, solitary or in pairs from small protuberances on the main trunk, green, mottled with white spots, and more or less pubescent with short, scattered hairs, the wall firm, 8 to 10 mm. thick; peduncles 1.5 cm. long, 4 mm. in diameter, pubescent, with three triangular, acuminate bracts at or slightly below the base of the receptacle; umbilicus 5 mm. in diameter, the protecting scales exerted 1 to 2 mm. Male flowers very numerous over the whole of the inside wall of the receptacle, 10 to 14 mm. long; perianth 1 mm. long, brown, of three distinct pieces; filament yellow 3 mm. long; anther one, white, 1 mm. wide, 1.5 mm. long. Gall flowers 8 mm. long stipitate, the ovary red, 2.5 mm. long, the style terminal, 0.5 mm. long. Fertile female flowers not seen.

Type specimen No. 322, Forestry Bureau, collected by P. T. Barnes, Lamao, Province of Bataan, Luzon, February, 1904.

A very striking and characteristic species found in the hill forests at an elevation of 200 m., but not at all common, characterized especially by its habit and very large mottled receptacles.

FICUS ODORATA (Blanco). *Ficus hispida odorata* Blanco, Fl. Filip. ed. 1, 686; ed. 2, 476; ed. 3, 3:89; *Ficus pungens*, Naves, 1 c. ed. 3, t. 358; F.-Vill., Nov. App. 200. 1883, non Reinw. § *Eusyce*.

This very characteristic species is not at all related to *Ficus pungens* Reinw., to which it was reduced by Naves and Fernandez-Villar. It is especially characterized by peculiar, strongly inequilateral, very rough, fragrant leaves, which are very similar in shape to those of *Ficus semicordata* Miq. The dried specimens retain the fragrance for a considerable period. *Ficus odorata* is fairly well represented by t. 358 of the third edition of Blanco's "Flora de Filipinas," although the figure does not show the striking hispid character of the branches. This species is also represented by No. 1522, Merrill, Dinalupijan, Province of Bataan, Luzon, January, 1903; also by No. 620, Forestry Bureau, collected by Borden, Lamao River, Province of Bataan, Luzon, April, 1904. T., *Paquiling* (according to Blanco, *Agos-os*).

FICUS CONORA King, Ann. Bot. Gard. Calcutta, 1:103. pl. 131. 1888.

This species previously known from New Guinea and Ternate, is represented by No. 683, Ahern, Surigao, Mindanao, 1901, distributed as *Ficus glomerata* Blanco. V., *Amison*. No. 3044, Merrill, from Castillejos, Province of Zambales, Luzon, August, 1903, is a closely related species, but differs from *Ficus conora* in some essential characters.

OLACACEÆ.

STROMBOSIA DUBIA Vidal, Sinopsis, Atlas, 20, t. 30 f. D. 1883.

This species is not noted in Index Kewensis, and is undoubtedly identical with *Strombosia philippinensis* (Baill.) Vidal. Vidal's type specimens were from the Province of Bataan, Luzon, where the species is very common, his type probably being No. 183 of his distribution. It is represented by the following specimens, all from Lamao River, Province of Bataan, Luzon. Nos. 514, 522, 532, 533, 549, 558, 578, 591 and 607 Forestry Bureau, collected by P. T. Barnes, November, 1903, to March, 1904; Nos. 639, 660, and 661 Forestry Bureau, collected by T. E. Borden, April, 1904, and No. 2515 Merrill, June, 1903.

A small tree, very common in the hill forests, reaching a height of 20 m. or less, and a diameter of 30 cm., flowering from April to June. Universally known to the Tagalogs of Bataan as *Camayaauan*.

ANONACEÆ.

POLYALTHIA BARNESII Merrill, sp. nov. § *Monoon*.

Tree about 20 m. high, with broadly lanceolate, or somewhat oblanceolate, glabrous, acuminate leaves, the flowers solitary or in fascicles of two or three, axillary or extra axillary on the branchlets, or on the branches below the leaves. Branches light brownish gray, lenticellate, with few scattered hairs, the ultimate branchlets densely pubescent. Leaves 14 to 19 cm. long, 5.5 to 8 cm. wide, the apex shortly acuminate, the base acute, rarely somewhat rounded, the upper surface glabrous, shining, beneath scarcely shining, and when young, with pubescent midvein and nerves, becoming glabrous;

nerves 12 pairs, rather prominent beneath, the reticulations lax, rather obscure; petiole rugose, thickened, 5 to 8 mm. long, when young densely pubescent, becoming glabrous. Mature flowers open, 5 cm. in diameter. Calyx 6 mm. in diameter, densely rusty pubescent, 3 lobed, the lobes rounded. Corolla of 6 spreading petals in two series, the petals of the inner series similar in shape and size to those of the outer series; petals yellowish green, shading to reddish brown at the base, lanceolate, or linear lanceolate, blunt, 2 to 2.5 cm. long, 5 to 8 mm. wide, sparingly rusty pubescent throughout, except near the base on the outer surface, where the pubescence is very dense; pedicels slender, 1.5 to 2.5 cm. long, densely rusty pubescent, ebracteolate. Ovaries indefinite, 1 mm. long, each with a single erect basal ovule; style oblong, 1 mm. long. Stamens numerous, 1 mm. long. Fruit unknown.

Type specimen No. 596, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, March, 1904. A tree growing in the hill forests at an altitude of 100 m.

MITREPHORA FERRUGINEA Merrill, sp. nov. (*Mitrephora maingayi* Vidal, Synopsis, Atlas, t. 5. f. F. 1883, non Hook f. et Th.)

A tree 10 to 15 m. high, with ovate-lanceolate, acuminate, membranaceous, more or less pubescent deciduous leaves, and densely ferruginous-pubescent branchlets and fruits. Branches grayish, glabrous, the ultimate branchlets very densely ferruginous-pubescent. Leaves 8 to 22 cm. long, 3.5 to 8 cm. wide, tapering above to the usually slender acuminate, rarely somewhat acute apex, and below to the acute base, the upper surface ferruginous-pubescent only on the midrib, smooth and shining, the under surface and nerves with scattered, rusty or whitish stellate hairs, the midrib densely ferruginous-pubescent; nerves 10 to 15 pairs, prominent beneath, curved upward, the reticulations subprominent, subparallel, lax; petiole thick, 5 mm. long, densely ferruginous-pubescent. Flowers hermaphrodite, odorless, in fascicles of two or three, only one flower developing at a time, 3 to 4 cm. in diameter, the pedicel 5 mm. long, densely ferruginous-pubescent, with three small, ovate, pubescent bracts at about the middle. Sepals very broadly triangular, acute, 3 mm. long, 3.5 mm. wide, densely ferruginous-pubescent on the outside. Petals creamy white, greenish at the base, the three outer ones spreading, narrowly ovate or somewhat obovate, 2 cm. long, 8 mm. wide above, the apex abruptly but bluntly acuminate, densely ferruginous-pubescent on the outside, glabrous on the inside, except for a few scattered hairs near the base, the inner three petals about 1.5 cm. long, vaulted, connivent above, and with long, slender claws, more or less ferruginous-pubescent on the outside. Stamens numerous yellow, slightly exceeding 1 mm. in length, the anther cells concealed by the overlapping connectives. Ovaries few, pubescent, 1 mm. long, 8 ovuled. Immature carpels ellipsoid, densely ferruginous-pubescent throughout, the apex with a small lateral protuberance, 3 to 3.5 cm. long, 2 to 2.5 cm. in diameter, sessile in fascicles of from 3 to 8 fruits each, on a thickened, ferruginous-tomentose receptacle.

Specimens examined, all from the Island of Luzon. Bataan Province, Lamao River, No. 610, Forestry Bureau, collected by Borden, April, 1904 (flower); No. 372, Forestry Bureau, collected by Barnes, November, 1903



PLATE II. *PANDANUS LUZONENSIS* MERRILL, FRUIT.

(sterile); No. 61, Forestry Bureau, collected by Barnes, October, 1903 (fruit); No. 367, March, 1904 (fruit); No. 3728, Merrill, January, 1904 (fruit); Province of Zambales, near Subig, No. 382, Forestry Bureau, collected by Maule, March, 1904 (fruit); Province of Camarines Sur, Nos. 67 and 253, Ahern, 1902 (fruit).

A rather common and apparently somewhat widely distributed species, growing in the dry hill forests at an altitude of from 100 to 500 m. above the sea. T., *Dalinas*.

CYATHOCALYX GLOBOSUS Merrill, sp. nov.

A tree 25 to 35 m. high, with dark-colored branches, glabrous ovate-lanceolate, acute or slightly acuminate subcoriaceous shining leaves and globose carpels 3.5 to 4 cm. in diameter. Branches glabrous, but the tips densely rusty pubescent. Leaves 15 to 25 cm. long, 5 to 10 cm. wide, both surfaces entirely glabrous, shining, the base acute or rarely somewhat rounded; main nerves 11 to 13 pairs, ascending, prominent beneath, anastomosing near the margin, reticulations rather lax; petiole 2 cm. long, at first densely pubescent, becoming glabrous, dark colored. Inflorescence leaf opposed, the flowers pediceled, two or three together; pedicels 8 mm. long, densely rusty pubescent, with usually a single ovate, densely pubescent bract 2 mm. long. Calyx 4 to 5 mm. long, three lobed, the lobes broadly triangular, acute or very abruptly and obscurely acuminate, densely rusty pubescent. Petals thick, greenish, densely grayish pubescent on both surfaces, 1.5 to 2 cm. long or more, 2 mm. wide above, the dilated base 4 mm. wide, narrowly oblong, lanceolate, obtuse, the inner ones similar in shape and size to the outer ones. Style cylindrical, 2.5 mm. long, the stigma discoid. Ovary one; ovules few. Stamens indefinite, 1.5 mm. long. Fruit solitary, firm, glabrous, black when dry, the peduncle 2 cm. long. Seeds 3 to 6, in two rows, much compressed, ovate, obtuse, 2 cm. long, smooth and shining.

Type specimens No. 560, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, March, 1904 (flower); No. 489, Forestry Bureau, collected by Barnes, same locality, November 1903 (fruit); other specimens, same locality, Nos. 510 and 523, Forestry Bureau, collected by Barnes, November, 1903 (sterile); Nos. 622, 646, 657, 667, Forestry Bureau, collected by Borden, April, 1904 (flower); No. 2130, Merrill, Lagumanoc, Province of Tayabas, Luzon, April, 1903 (fruit).

A tree, apparently common in the hill forests, reaching a diameter of 50 cm. or less. T., *Latauan*.

UVARIA ALBA Merrill, sp. nov.

An erect shrub about 3 m. high with leaf opposed, 2 to 3 flowered inflorescence, white flowers, oblong-lanceolate, acuminate leaves, the young branches, leaves, and inflorescence densely covered with a rufous pubescence. Leaves 15 to 22 cm. long, 6 to 10 cm. wide, widest in the upper third, from this point tapering gradually to the broad, abruptly rounded base, the apex rather abruptly and narrowly acuminate, the acumen 2 to 3 cm. long, very rarely nearly acute, the upper surface with few scattered, spreading rufous hairs, the nerves and midrib densely rufous pubescent,

the under surface rather densely pubescent throughout with spreading rufous hairs, the nerves and midrib especially densely pubescent; nerves rather prominent, 16 to 18 pairs, anastomosing near the margin, the reticulations nearly obsolete above, prominent on the lower surface; petioles 1 to 1.5 cm. long, densely rufous pubescent. Inflorescence leaf-opposed, 2 to 3 flowered, 4 to 5 cm. long, densely rufous pubescent; peduncles 1.5 cm. long, the bract situated at the upper third, clasping, rufous pubescent, 5 mm. long, 11 mm. wide. Flowers 3 to 3.5 cm. in diameter, white, odorless. Sepals densely rufous puberulent, free or slightly connate at the base, similar to the bract in shape and size. Petals somewhat fleshy when fresh, ovate, obtuse, densely rufous puberulent, 12 to 15 mm. long, 8 mm. wide. Stamens indefinite, 6 mm. long, the outer ones flat. Carpels ovate, 2.5 cm. long, 12 mm. in diameter, densely pubescent with somewhat stellate, rufous hairs.

Type specimen No. 50, Forestry Bureau, collected by P. T. Barnes, Lamao River, Province of Bataan, Luzon, August 11, 1903, in flower; No. 3274, Merrill, from the same locality, October 1903, with flower buds, and fruits is the same. *T. Susu-calabao*.

This species is evidently related to *Uvaria hamiltoni* Hook. f. et Th., from British India, but is entirely distinct. Its striking characteristics are its peculiarly shaped leaves, white flowers, and uniform rufous pubescence.

HERNANDIACEÆ.

ILLIGERA LUZONENSIS (Presl). (*Henschelia luzonensis* Presl, Rel. Haenk. 2:81. t. 63. 1831; *Illigera meyeniana* Kunth, ex Walp. in Nov. Act. Nat. Cur. 19. Suppl. 1:410. 1843; *Illigera appendiculata* Vidal, Synopsis Atlas, t. 48. f. F., non Blume; *Gronovia ternata* Blanco, Fl. Filip. ed. 1, 186. 1837; *Halesia ternata* Blanco, l. c. 399.)

This species is represented in the herbarium by the following numbers, all from Luzon. Province of Rizal, Antipolo, No. 1683, Merrill, March, 1903; Province of Bataan, Lamao River, No. 2557, Merrill, June, 1903; No. 3289, Merrill, same locality, October, 1903.

Presl's name is the earliest for this species and should be taken up. *Gronovia ternata* Blanco, was based on a specimen collected at Malinta, near Manila, and is undoubtedly this species. *Halesia ternata* Blanco, although published in the same work as the preceding species, is without doubt identical. Blanco described the latter from a specimen in fruit only, collected in Angat, Province of Bulacan, Luzon. Blanco gives no native names, but the specimen from Antipolo cited above, bears the Tagalog name *Saling ouac*.

ROSACEÆ.

PHOTINIA LUZONENSIS Merrill, sp. nov.

A small tree, 3 to 6 m. high, with ovate or ovate-lanceolate, acute, serrate leaves, and terminal, corymbose, ferruginous-pubescent inflorescence. Branches gray, glabrous, the ultimate branchlets ferruginous-pubescent. Leaves 5 to 10 cm. long, 1.5 to 4 cm. wide, the base cuneate and somewhat

decurent, the apex acute, margins serrate in the upper half or two-thirds, entire near the base, subcoriaceous, glabrous and shining above, the under surface with rather prominent brown reticulations, entirely glabrous, or the midrib ferruginous-pubescent in young leaves; nerves about 13 pairs, obscure above, fine, but prominent beneath, brown; petiole about 1 cm. long, ferruginous pubescent, becoming glabrous. Inflorescence a terminal corymbose panicle 2.5 to 7 cm. long, the axis and branches densely ferruginous-pubescent, the branches few, the lower ones, in larger corymbs, 5 cm. long; bracts linear, acuminate, 5 mm. long, the bracteoles 2 mm. long. Flowers white, odorless, 10 to 12 mm. in diameter, the pedicels 4 to 5 mm. long, densely ferruginous-pubescent. Calyx obconic, densely ferruginous-pubescent, the teeth triangular, acute, nearly 2 mm. long. Petals obovate, glabrous, 5 mm. long, 4 mm. wide, the base unequal, reticulate. Stamens many, filaments slender, glabrous, 2 mm. long; anthers 0.5 mm. long. Ovary with a ferruginous-pubescent crown, 2 celled, each cell with two erect ovules. Styles 2, free, slender, glabrous, nearly 3 mm. long.

Type specimen No. 3223, Merrill, Mount Mariveles, Province of Bataan, Luzon, October, 1903; No. 3714, Merrill, from the same locality, January 1, 1904, is the same.

A small tree 3 to 6 m. high, with a trunk diameter of from 20 to 30 cm., growing on exposed wind-swept ridges at an elevation of 1,200 to 1,300 m. above the sea.

PARINARIUM RACEMOSUM Merrill, sp. nov.

A tree 22 m. high, with lanceolate or oblong-lanceolate, nearly glabrous, acuminate, short-petioled leaves, racemose inflorescence, the flowers with 23 stamens. Branchlets dark gray, or nearly black when dry, glabrous. Leaves subcoriaceous, 10 to 13 cm. long, 3 to 5 cm. wide, tapering above to the short, acuminate apex, the base rounded, slightly inequilateral, the upper surface shining, glabrous except for a few scattered hairs on the midrib, the reticulations and veins with numerous, minute papillæ, giving them a beaded appearance under the lens, the under surface glabrous; nerves 10 to 12 on each side of the midrib, not prominent, ascending, reticulations rather dense, the midrib with two glands near the base of the leaf; petioles 4 mm. long, glabrous or with few appressed hairs. Racemes 3 to 8 cm. long, solitary or two or three from the same leaf axil, densely pubescent. Flowers, including the stamens, 2.5 cm. long, the pedicels 3 mm. long. Bracts 7 cm. long, 3 mm. wide, acute, densely pubescent throughout, deciduous. Calyx tube funnel shaped, 8 mm. long, densely pubescent outside, deflexed-villous inside, the lobes 6 to 8 mm. long, 3 to 4.5 mm. wide, acute. Petals 8 to 10 mm. long, 4 to 5 mm. wide, glabrous, obtuse, the base subacute. Stamens 23, the filaments about 2 cm. long, united at the base and somewhat pubescent below. Ovary densely villous, 2 celled. Style filiform, 2 cm. long, densely villous below, glabrous above. Fruit unknown.

Type specimen No. 2416 Merrill, Marintoc River, Island of Masbate, May 17, 1903. A tree in forests along the river slightly above sea level known to the Visayans as *Tambontambon*.

A species evidently closely related to *Parinarium scabrum* Hassk.

LEGUMINOSÆ.

INTSIA ACUMINATA Merrill, sp. nov.

A tree with 4-jugate leaves, the leaflets coriaceous, glabrous, bluntly acuminate. Branches gray, glabrous. Leaf rachis glabrous, 8 cm. long, the internodes 2 cm. long; leaflets broadly ovate, opposite, the base slightly unequal, rounded, the apex rather abruptly tapering to the short, blunt, often emarginate acumen, 5 to 6.5 cm. long, 2.5 to 3.5 cm. wide, coriaceous, shining, entirely glabrous except for the midrib beneath, which is often densely rusty pubescent; petioles thick, glabrous, 2 mm. long; nerves numerous, not prominent, reticulating. Flowers unknown. Pod 15 cm. long, 5.5 cm. wide, glabrous, firm, the apex with a short, sharp, slightly curved acumen.

Type specimen, No. 1108, Merrill, Baler, Province of Principe, Luzon, October, 1903. Locally known as *Tindalo*, but this name is almost invariably applied to *Pahudia rhomboidea* (Blanco) Prain.

This species undoubtedly belongs in the genus *Intsia*, although at present the flowers are unknown. It differs from *Intsia bijuga* (Colebr.) O. Kuntze, in its much smaller, coriaceous, more numerous leaflets, and from both *Intsia bakeri* Prain, and *Intsia palembanica* Miq., in its smaller differently shaped leaflets and other characters.

PTEROCARPUS ECHINATUS Pers. Synopsis, 2:277. 1807. (*Pterocarpus erinaceus* F.-Vill., Nov. App. 68. 1880; Vidal, Synopsis, Atlas, t. 40. f. B. 1883, non Lam.; *Pterocarpus vidalianus* Rolfe, Journ. Linn. Soc. Bot. 21:309. 1884; Vidal, Rev. Pl. Vasc. Filip. 112. 1886; Perkins, Frag. Fl. Philip. 20. 1904.)

The type locality for this species as given by Persoon is "Hab. in India prope Capo de Solar," and in compiling Index Kewensis, the authors of that work could not definitely determine this locality, and accordingly questionably referred *Pterocarpus echinatus* to tropical Africa. It remained for Dr. D. Prain¹ to determine the geographical locality of "Capo de Solar," which is a small island in the Flores Sea, south of Celebes, "Selayar" or "Salajar" of modern maps. Dr. Prain had a specimen of *Pterocarpus echinatus* Pers., from a tree cultivated in the Botanical Garden at Buitenzorg, Java, which was raised from seed collected by Zollinger at Macassar, southern Celebes, on the northern shore of the Flores Sea. This specimen Dr. Prain sent to Kew, and Sir George King compared it with the type of *Pterocarpus vidalianus* Rolfe, and found the two species to be identical.

The establishment of the identity of *Pterocarpus echinatus* and *P. vidalianus* adds another point to our knowledge of the close relationship of the flora of Celebes to that of the Philippines, at the present time 60 or more species being known only from Celebes and the Philippines.

Pterocarpus echinatus is a common species in the Philippine forests but apparently does not extend north of central Luzon (Baler, Province of

¹ Report on the Indian Species of *Pterocarpus*, Indian Forester, 26:10. 1900.

Principe, No. 1016, Merrill). It is especially abundant in the central portions of the Archipelago, but has as yet not been found in Paragua. The natives do not distinguish this species from *Pterocarpus indicus* Willd., both being known as *Narra* or *Asana*.

BAUHINIA PERKINSÆ Merrill, sp. nov. § *Phanera*.

A woody, climbing vine, 4 to 5 m. long, with large cordate leaves which are ferruginous-pubescent beneath, and with rounded apical lobes, ample racemose inflorescence, the petals glabrous or nearly so. Branches slightly pubescent, becoming glabrous. Leaves membranaceous, roundish, 12 to 14 cm. long, 14 to 17 cm. wide, the base strongly cordate, the basal lobes rounded, apex cleft, the sinus 3 to 4 cm. deep, narrow, the lobes somewhat overlapping below, the midrib slightly excurrent, glabrous and shining above, or with few scattered hairs near the insertion of the petiole, the pubescence beneath especially prominent on the nerves and reticulations; nerves 13, prominent beneath, the reticulations lax; petiole densely rusty pubescent, 6 cm. long. Racemes 20 cm. long or more, 10 cm. across, the pedicels spreading, 3.5 to 4 cm. long, densely rusty pubescent. Buds acute, club shaped, 2.5 cm. long, the slender basal portion 5 to 8 mm. long; calyx very densely rusty pubescent, the limb splitting into five reflexed, lanceolate, acuminate segments, 2 cm. long, 4 mm. wide, the tube slightly enlarged toward the base, 8 mm. long. Petals five, subequal, broadly oblanceolate, abruptly acute or apiculate, 2.5 cm. long, 8 mm. wide, the claw 5 mm. long, glabrous throughout, or with very few scattered hairs on the outside near the apex. Stamens 3, fertile, the filaments nearly 2 cm. long, the anthers 1 cm. long. Ovary stalked, densely rusty, silky-pubescent, the style stout, rusty-pubescent, 5 mm. long.

Type specimen No. 731, Merrill, Ewiig River, near Puerto Princesa, Paragua, February 15, 1903, a vine growing in rather dry thickets along the river, near sea level.

This species was determined by Perkins¹ as *Bauhinia ferruginea* Roxb., but it certainly can not be that species, differing in its longer petioles, larger leaves, more numerous nerves, and glabrous, or nearly glabrous, not tomentose, petioles.

RUTACEÆ.

CLAUSENA ANISUM-OLENS (Blanco). *Cookia anisum-olens* Blanco, Fl. Filip. ed. 1, 359. 1837; *Cookia anisodora* Blanco, l. c. ed. 2, 253. 1845; *Clausena excavata* F.-Vill., Nov. App. 36. 1880, non Burm.; *Clausena indica* Vidal, Sinopsis, Atlas, t. 25. f. H. 1883, non Oliv.

This species is represented in the herbarium by No. 2509, Merrill, Lamac River, Province of Bataan, Luzon, June, 1903, and is apparently very distinct from *Clausena excavata* Burm., to which it has previously been referred. F.-Villar referred Blanco's species to *Clausena excavata* without comment, although Blanco distinctly states that the flowers of his species were five parted. *Clausena anisum-olens* can be distinguished from *C. exca-*

¹ Frag. Fl. Philip. 9. 1904.

vata, not only by its five-parted flowers, but also by its less numerous and differently shaped leaflets, the leaflets being usually of but four pairs, from 3 to 4 cm. wide, the base more or less unequal, rounded, scarcely narrowed. Vidal in preparing his Sinopsis evidently noted Villar's error in the identification of *Clausena excavata*, and identified the same form as *Clausena indica* Oliv., his figure representing a five-parted flower. *Clausena excavata* Burm., is reported from the Philippines by Vidal,¹ Nos. 142, 1217, and 144, and also has been collected by Loher, No. 196, the identifications in both cases having been made at Kew. Careful examination of this material will show whether or not these numbers represent true *Clausena excavata*, or the species here considered. *Clausena anisum-olens* Blanco is known to the Tagalogs as *Calomata*, *Camanguianis*, and *Maisipaisi*. The leaves are very aromatic when crushed.

MELIACEÆ.

AGLAYA BORDENII Merrill, sp. nov. § *Euaglaia*.

A small tree, 12 m. high or less, with bijugate, glabrous or nearly glabrous leaves, and many-flowered panicles, which are about one-half as long as the leaves, or less, with small, very fragrant yellow flowers. Branches glabrous, light gray, the tips more or less brown stellate-pubescent. Leaves alternate, 20 cm. long or less, the rachis 8 cm. long, grayish or brownish stellate-pubescent; leaflets 8 to 11 cm. long, 3 to 4.5 cm. wide, ovate-lanceolate, membranous, the apex slender acuminate, the base cuneate, nearly equilateral, both surfaces glabrous, the midrib beneath with few scattered stellate hairs, the lower leaflets slightly smaller than the upper, and the terminal leaflet about the same size or slightly larger than those of the upper pair; nerves about 10 pairs, rather prominent; petiolules, 3 mm. long, very densely clothed with brown stellate hairs. Panicles 10 cm. long or less, axillary, branched from the very base, the lower branches 4 to 7 cm. long, spreading, the branchlets 2 cm. long or less, the entire inflorescence, excepting the flowers, densely brown stellate-pubescent. Flowers yellow, pedicellate, broadly obovoid or subglobose, 2 mm. long, the pedicels 1 mm. long. Calyx deeply 5 toothed, the teeth broadly triangular, 0.5 mm. long. Petals five, free, elliptical, glabrous, obtuse, 2 mm. long, 1.5 mm. wide. Staminal tube 1.5 mm. long, truncate, abruptly widened at about the middle, the five stamens inserted on the tube just above the middle. Anthers ovate, acute, 0.6 mm. long, included, or the very tips exerted.

Type specimen No. 714 Forestry Bureau, collected by T. E. Borden, Lamao River, Province of Bataan, Luzon, May, 1904, also No. 631 Forestry Bureau, collected by Borden, same locality, April, 1904.

A small tree 12 m. high or less, and 20 cm. in diameter or less, branching at a height of from 6 to 8 m., with slightly rough, grayish bark, and reddish-yellow wood, growing on forested ridges, at an elevation of 160 m. above the sea. Tagalog, *Potian*.

¹ Rev. Pl. Vasc. Filip. 77

LANSIUM DUBIUM Merrill, sp. nov.

A shrub or small tree with unequally pinnate leaves, glabrous entire leaflets and slender, glabrous, elongated, few-flowered axillary racemes. Branches dark colored, glabrous. Leaves 20 cm. long, the rachis about 8 cm. long, slender, glabrous, even or odd pinnate, the leaflets usually alternate, rarely opposite, 4 or 5 on each leaf; leaflets ovate-lanceolate, bluntly acuminate, glabrous, tapering to the cuneate or somewhat decurrent, often slightly unequal base, above rather abruptly contracted to the stout, blunt acumen, 8 to 15 cm. long, 2.5 to 5 cm. wide, the nerves very numerous, not prominent, spreading, the primary nerves scarcely more prominent than the secondary ones; petiolules proper, stout, glabrous, 2 mm. long. Racemes slender, usually about as long as the leaves, the flowers scattered, sessile or subsessile. Flowers glabrous, subglobose, yellow, 5 mm. long. Sepals 5 orbicular, rounded, imbricate 2 mm. long. Petals 5, free, imbricate, broadly ovate or obovate, the apex obtuse, 5 mm. long. Staminal tube 4 mm. long, 10 toothed, the teeth acute, less than 0.5 mm. long. Stamens 10, in two series, five inserted in the middle of the tube, included, and five inserted near the tips of alternating teeth of the tube, their apices somewhat exerted, the anthers of the first series 2 mm. long, those of the second series 1.5 mm. long. Ovary conical about 1 mm. long, pubescent, 4 celled, each cell with a solitary axial ovule. Disc inconspicuous or wanting. Stigma sessile. Fruit globose, brown, indihiscent, the pericarp rather thin, brittle, dry, one celled and with a single large seed, the cotyledons very thick, exalbuminous.

Type specimen No. 3081, Merrill, Island of Masbate, August, 1903. A characteristic species, differing from the other species in the genus especially in its fruit characters, but apparently a species of the genus.

AMOORA LEPIDOTA Merrill, sp. nov.

A tree 20 to 30 m. high with pinnate leaves and axillary and terminal panicles of yellow fragrant flowers, the petioles, inflorescence, and young branches being densely clothed with small brownish-ciliate scales. Leaves 2 to 4 dm. long, alternate, the petiole 1 to 2.5 dm. long. Leaflets 4 to 7, alternate, 8 to 20 cm. long, 3 to 7 cm. wide, ovate-lanceolate, the base cuneate, the apex acuminate, glabrous above, or with few scattered scales when young, beneath densely and minutely punctate, and with scattered scales especially on the midrib and nerves; veins about 11 pairs, rather prominent beneath; petiolules densely scaly 0.5 cm. long. Panicles 1 to 2 dm. long including the peduncle, which is from 3 to 8 cm. to the first branches, 10 to 15 cm. wide, much branched, the branches spreading, 3 to 8 cm. long. Flowers numerous, subglobose, 3 mm. long, with a powerful but somewhat disagreeable odor; pedicels densely scaly, 2 to 4 mm. long. Calyx 1.5 mm. long, densely scaly, 5 toothed, the teeth acute, 0.5 to 0.8 mm. long. Corolla glabrous, yellow; petals 5 broadly ovate or ovate lanceolate, concave, obtuse, 3 mm. long, free from the staminal tube, or very slightly joined at the base. Staminal tube equaling the corolla. Stamens 10, included, anthers 1 mm. long. Ovary pubescent 0.5 mm. long; style very short, 0.2 mm. Stigma less than 0.5 mm. long.

Type specimen No. 3173, Merrill, hill forests along the Lamao River, Mount Mariveles, Province of Bataan, Luzon, October, 1903; altitude 100 m.

A species evidently related to *Amoora maingayi* Hiern, from Malacca but distinct from the latter. It is characterized by its inflorescence, petioles, etc., being covered with numerous small scales, the margins of which are lacinate or ciliate, giving the scales a stellate appearance. This species belongs in the section *Pseudo-aglaia*.

AMOORA AHERNIANA Merrill, sp. nov.

A tree 30 m. high, glabrous throughout, with deep-brown branches and inflorescence, alternate, 9 to 10 jugate leaves, and short, axillary, racemose inflorescence. Ultimate branches thickened, 1 cm. in diameter, rugose, the leaf scars very large. Leaves unequally pinnate, crowded at the ends of the branches, about 60 cm. long, the rachis stout, deep brown, glabrous, the petiole 15 cm. long; leaflets opposite, coriaceous, 10 to 15 cm. long, 4 to 5 cm. wide, the upper surface shining, ovate-lanceolate, acuminate, glabrous, brownish when dry, inequilateral, usually somewhat falcate, the base unequal, rounded or somewhat acute, the nerves prominent, 10 to 12 on each side, spreading, curved upward, the reticulations rather lax; petiolules 10 to 15 mm. long, deep brown. Racemes axillary, 5 cm. long (with immature flowers), deep brown, glabrous, striate. Flowers (immature) with a stout deep-brown pedicel, 5 mm. long. Calyx glabrous, reddish brown, subglobose, 4 mm. in diameter, with three, sometimes four, broad, rounded teeth. Petals three, broadly ovate concave, glabrous, obtuse, 6 mm. long, 4 to 5 mm. wide. Staminal tube, 4.5 mm. long, with 10 small blunt teeth. Stamens 9 to 13, included, sessile, the anthers 2.5 mm. long. Disc wanting. Ovary 4 celled, each cell with 2 superposed ovules. Style none. Stigma pyramidal, reddish, 1 mm. long. Fruit globose, glabrous, bright red, three celled, dehiscing by three valves, 6 cm. in diameter, the pericarp spongy when fresh, 1 to 1.5 cm. thick. Seeds one in each cell, or sometimes one or more aborted, 3 cm. long, 1 cm. thick, smooth, flattened, the arillus thick, yellowish, surrounding less than one-half of the seed.

Type specimen No. 823 Forestry Bureau, collected by T. E. Borden, Lamao River, Province of Bataan, Luzon, June, 1904. A tree reaching a diameter of 45 cm., growing on forested ridges at an elevation of 600 m., with reddish-brown bark, and reddish wood, the resinous pericarp of the fruit being somewhat used by the natives for illuminating purposes. Tagalog, *Cato*.

This species is somewhat anomalous, but is apparently a good species of *Amoora*, differing from other species in the genus by the variable number of calyx teeth, variable number of stamens, and four-celled ovary. This distinct species is named in honor of Capt. G. P. Ahern, Chief of the Forestry Bureau.

AMOORA MACROCARPA Merrill, sp. nov.

A small tree 8 m. high, with very short, spicate, axillary inflorescence, and large spherical fruits. Young branches minutely pubescent, becoming glabrous. Leaves alternate, unevenly pinnate, 3.5 to 4.5 dm. long, the rachis minutely pubescent, becoming glabrous; leaflets 4 to 5 on each side, alter-

nate, ovate, 12 to 20 cm. long, 6 to 8 cm. wide, the apex acuminate, the base rounded or acute, more or less irregular, nearly glabrous above, minutely lepidote beneath; nerves rather prominent, about 12 pairs; petiolules 5 to 10 mm. long. Inflorescence axillary, spicate, the spikes 1 to 2 cm. long, the axis densely rusty velvety pubescent, 3 mm. in diameter. Flowers sessile, ovate, 5 to 6 mm. long. Calyx truncate, 3 to 4 mm. long, obscurely and broadly three toothed, densely and minutely pubescent. Petals white, three, broadly ovate, 5 mm. long, 3 mm. wide, acute or nearly obtuse, densely and minutely rusty tomentose. Staminal tube cylindrical 3.5 mm. long, glabrous, the margin irregularly and obscurely toothed, the teeth truncate. Stamens 7, the anthers sessile on the upper half of the staminal tube, 1.5 mm. long, included. Disk annular, 1 mm. long, glabrous, truncate. Ovary subglobose, pubescent, three celled, each cell with two ovules. Style 2 mm. long; stigma subglobose. Fruit spherical, 5 to 6 cm. in diameter, brown, minutely and densely velvety pubescent, 2 or 3 celled, with one large seed in each cell, the pericarp subcoriaceous, brittle, thin. Seeds 3.5 cm. long, about the same width and 2.5 cm. thick, the testa coriaceous, tough. Cotyledons about 2 cm. thick, the radicle included.

Type specimen No. 3731, Merrill, Mount Mariveles, Province of Bataan, Luzon, January 1, 1904. A small tree 8 m. high with a trunk diameter of about 10 cm., not common on dry, wooded slopes at an elevation of 600 m.

This plant is undoubtedly a species of *Amoora*, as that genus is treated by C. de Candolle¹ belonging to the section *Pseudo-guarea*. Some of the floral characters of this species, the short inflorescence, the disc, and the obscurely three-toothed calyx, do not agree well with the generic description of *Amoora*, but as the plant is nearest to this genus, it is so referred.

DYSOXYLUM ALTISSIMUM Merrill, sp. nov. § *Eudysosyllum*.

A tree 25 m. high or less, with alternate, glabrous, bijugate or trijugate, odd or even pinnate leaves, many-flowered panicles, half as long as the leaves or less, and creamy white, fragrant flowers 13 mm. long. Branches brownish or brownish gray, rugose, glabrous. Leaves 30 to 40 cm. long, the leaflets three pairs, and even pinnate, or two pairs and odd pinnate by suppression of one of the leaflets of the terminal pair; petiole 7 to 10 cm. long, the rachis with the petiole 13 to 20 cm. long; leaflets opposite or subopposite, ovate-lanceolate, or elliptical-lanceolate, subcoriaceous, glabrous, the upper surface shining, the apex acute, the base acute, inequilateral 11 to 19 cm. long, 5 to 7 cm. wide, the nerves 10 to 11 on each side, curved upward, not prominent, the reticulations nearly obsolete; petiolules rugose 10 to 15 cm. long. Panicles 20 cm. long or less, the branches spreading, the lower ones 8 cm. long or less. Flowers about 13 mm. long, the pedicels 3 mm. long or less, slightly pubescent. Calyx 2 mm. long, reddish brown, pubescent with few scattered hairs, with four short, acute teeth. Petals four, 12 mm. long, 4 mm. wide, the apex bluntly acute, minutely appressed-pubescent outside, the inside glabrous. Staminal tube cylindrical, 9 mm. long, glabrous, the margin entire. Stamens eight, inserted near the apex of the tube, included, the anthers 1.2 mm. long. Disk cylindrical, 4 mm.

¹ Monog. Phan. 1:579.

long, the margin ciliate. Ovary pubescent, four celled. Style elongated, glabrous, swollen at the apex, the tip just exerted from the staminal tube. Stigma discoid, 1.3 mm. in diameter. Fruit unknown.

Type specimen No. 624, Forestry Bureau, collected by T. E. Borden, Lamao River, Province of Bataan, Luzon, April, 1904. A tree reaching a diameter of 70 cm., with brownish-red bark, growing in forests at an altitude of about 100 m. Tagalog, *Guso*.

EUPHORBIACEÆ.

ANTIDESMA EDULE Merrill, sp. nov.

A shrub or small tree 3 to 18 m. high, with ovate-lanceolate or broadly ovate, acute or acuminate, membranaceous, more or less pubescent, shining leaves, the inflorescence in small axillary panicles, 4 cm. long or less. Branches reddish brown, densely pubescent throughout. Leaves 10 to 22 cm. long, 4.5 to 9.5 cm. wide, the base rounded or subacute, often slightly unequal, the apex acute or more often acuminate, the upper surface more or less pubescent, becoming glabrous, shining, the under surface paler than the upper, uniformly pubescent with scattered hairs, the midrib shining and veins densely pubescent; nerves about ten pairs, ascending, obscure above, rather prominent beneath, the reticulations lax; petioles 0.8 to 2 cm. long, densely pubescent. Panicles axillary, the rachis and branches densely tomentose, the branches few, ascending, 3 cm. long or less; bracts lanceolate, acuminate, densely hirsute, 3.5 mm. long; bracteoles ovate-lanceolate, subhyaline, ciliate, 1 mm. long. Male flowers numerous, the calyx 1 mm. long, clothed with scattered white hairs, very obscurely five toothed or nearly entire, ciliate. Stamens 4; filaments glabrous, 2 mm. long. Pedicel thick, 1 mm. long or less. Female flowers not known. Fruit ellipsoid or subglobose, 3.5 to 4 mm. in diameter, pink or red, edible, with a pleasant acid taste, the style persistent near the apex, lateral, the persistent calyx five-lobed, the lobes scarcely reaching the middle of the calyx.

This species is represented by the following specimens, all from Lamao River, Province of Bataan, Luzon: No. 3148, Merrill; October, 1903 (fruit); No. 3784, Merrill, January, 1904 (immature flowers); No. 167, Forestry Bureau, collected by Barnes, January, 1904 (male flowers); No. 574, Forestry Bureau, collected by Barnes, March, 1904 (fruit), the latter number with narrow, very acuminate leaves.

A shrub or small tree growing in the hill forests at an elevation of about 100 m. T., Pamp., *Tanigi*.

CYCLOSTEMON BORDENII Merrill, sp. nov. (*Cyclostemon macrophyllus* Vidal, Sinopsis, Atlas, t. 82 f. B. 1883, non Blume.)

A tree 23 m. high or less, with oblong-ovate or oblong-lanceolate, coriaceous, entire, glabrous leaves, the flowers in axillary fascicles, or on the branches below the leaves. Branches light gray, the tips usually brownish. Leaves 10 to 23 cm. long, 4 to 7 cm. wide, those of the sterile branches 20 to 40 cm. long, 6 to 11 cm. wide, smooth and shining on both surfaces, the apex acute or slightly acuminate, the base very unequal, one side rather broadly rounded, the other very acute; nerves 6 to 9 pairs, ascending, curved, rather prominent, freely anastomosing, the reticulations rather

prominent, lax; petioles thickened, rugose, 6 to 9 mm. long; stipules narrowly lanceolate-acuminate, somewhat pubescent. Male flowers globose, about 6 mm. in diameter, short pediceled, 6 to 10 in a fascicle, the pedicels 3 mm. long or less, densely pubescent; sepals four, coriaceous, concave, orbicular, entire, about 6 mm. in diameter, the outside densely pubescent with short, appressed grayish-brown hairs, the inside glabrous. Stamens many, the anthers 2 mm. long; disk broad, glabrous. Female flowers unknown. Fruit subglobose, 1.5 cm. in diameter, gray, pubescent, becoming glabrous, 2 celled, each cell with a single large seed.

Specimens examined: Nos. 333 (type), 542, 563, 573 Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, 1904; Nos. 671 and 673 Forestry Bureau, collected by T. E. Borden, same locality, May, 1904, also No. 11 Ahern, Pasacao, Province of Camarines Sur, Luzon, January, 1902 (flower). Of the specimens collected by Barnes, No. 333, February, 1904, only is in flower.

This species is rather common in the hill forests at Lamao, at an elevation of about 100 m. above the sea, reaching a diameter of 30 cm. or less, and a height of 23 m. or less. Tagalog *Diladila* or *Talimorung*.

CYCLOSTEMON MICROPHYLLUS Merrill, sp. nov.

A tree 16 m. high or less, with glabrous ovate or ovate-lanceolate inequilateral, acuminate leaves, and fasciculate axillary inflorescence. Branchlets slender, light gray, glabrous. Leaves 4 to 7 cm. long, 1.5 to 3.5 cm. wide, subcoriaceous, glabrous, shining, the base unequal, rounded or subacute, tapering above to the acuminate apex, the nerves four on each side, ascending, curved, obscure above, not prominent beneath, anastomosing, the reticulations obscure, lax; petioles 4 to 5 mm. long, slightly pubescent at first, becoming glabrous. Flowers in fascicles of ten or less in the axils of leaves or of fallen leaves, greenish white, odorless, globose, 4 mm. in diameter, the pedicels slender, slightly pubescent 4 to 5 mm. long. Sepals four, sparingly rusty-pubescent, imbricate, broadly ovate or suborbicular, 4 mm. long. Stamens 9 to 12, the filaments 1 mm. long, the anthers 1.7 mm. long. Female flowers and fruit unknown.

Type specimen No. 296, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, January, 1904. A small tree, 16 m. high or less and 25 cm. in diameter or less, in the hill forests at an altitude of 150 m. above the sea. Tagalog, *Tangnaranig*.

ANACARDIACEÆ.

MANGIFERA ALTISSIMA Blanco, Fl. Filip. ed. 1, 181. 1837; ed. 2, 129; ed. 3, 1:230.

This species was reduced by Fernandez-Villar¹ to *Mangifera longipes* Griff., which is certainly an error. *Mangifera altissima* Blanco is very closely related to, if not identical with, *Mangifera quadrifida* Jack, a species of the Malayan Peninsula. Engler² retains Blanco's species among the uncertain species. The vegetative and floral characters of *Mangifera altissima* Blanco agree very well with those of the published descriptions of

¹ Nov. App. 54. 1880.

² Monog. Phanerog. 4:214.

Mangifera quadrifida Jack, but the fruit of the latter is very imperfectly known, and is described as subglobose, which does not apply to Blanco's species. The fruit of *Mangifera altissima* strongly resembles that of *Mangifera indica* Linn., in form, but is much smaller and not as much compressed. It is smooth and green or somewhat yellowish when ripe, ovoid or ellipsoid, rounded at both ends, slightly compressed, 5.5 to 8 cm. long, 4 to 6 cm. thick, the fleshy pericarp rather firm, white, not at all stringy. Frequently the point of insertion of the style persists in the ripened fruit as a small protuberance on the side near the apex. Seed similar in shape to the fruit, glabrous, not stringy, 3 to 5.5 cm. long, 3 to 4 cm. thick.

There are apparently two forms of this species, as noted by Blanco, the one here described being that noted by Blanco under the native name *Pahohotan*, the fruits having a very strong flavor of turpentine. Possibly the form noted by Blanco under the native name *Paho*, may be distinct, but no specimens have been seen, the ones cited below bear the three native names, *Paho*, *Pahutan*, and *Pahohotan*.

Specimens examined: Luzon—Province of Zambales, Subig, No. 1757, Merrill, April, 1903; Province of Bataan, Lamao, Nos. 256, 484, 485, 487, 502, Forestry Bureau, collected by Barnes, March 1904; Nos. 642, 643, Forestry Bureau, collected by Borden, April, 1904, same locality; No. 3807 Merrill, March, 1904, same locality; Province of Rizal, Antipolo, No. 51, Decades Philippine Forest Flora, collected by Ahern's collector, February, 1904. Mindoro—Pola, No. 2366, Merrill, May, 1903; Calapan, without collector or date. A common tree, flowering in February and March, fruiting in March, April, and May.

MANGIFERA MONANDRA Merrill, sp. nov.

A medium-sized tree, entirely glabrous, with coriaceous, lanceolate, broadly lanceolate or sometimes obovate-lanceolate, acute or very shortly and bluntly acuminate, rather long petioled leaves, small panicles, 10 cm. long or less, and small flowers, 3.5 mm. long. Branches grayish brown, glabrous, not thickened. Leaves 9 to 16 cm. long, 2.5 to 6.5 cm. wide, narrowed to the cuneate base, the upper surface shining, the nerves 11 to 13 on each side, rather distinct, at intervals of about 1 cm., the reticulations beneath, fine, distinct, nearly obsolete above; petioles thickened and rugose at the base, 2.5 to 4 cm. long, those of the upper and smaller leaves shorter. Panicles glabrous, 10 cm. long or less, two or three from the tip of each branchlet, the branches slender, the lower ones 3.5 cm. long or less, the panicle in fruit elongated to 20 cm. Flowers white, the pedicels 1 to 2 mm. long. Sepals 4, broadly ovate, 2 to 2.5 mm. long, 1.5 mm. wide, thin, acute, three nerved. Petals 4, broadly ovate, obtuse, the veins 5 to 7, prominent as thickened, confluent ridges below, slender and branched above, not extending to the margins. Disk thickened, nearly 2 mm. wide, and 1 mm. thick. Fertile stamen one, the filament about 1 mm. long, the anther 0.8 mm. long; staminodes three, very minute, or obsolete. Ovary globose, glabrous, the style sublateral, 1.5 mm. long. Fruit, ellipsoid, somewhat inequilateral, subcompressed, 3.5 cm. long, 1.8 cm. wide, 1.5 cm. thick, the pulp very thin.

Type specimens No. 414, Forestry Bureau, collected by Ahern's collector, Antipolo, Province of Rizal, Luzon, February, 1904 (flower). No 441, same collector and locality, April, 1904 (fruit). A tree growing in the forests. Tagalog, *Clamansane*.

STERCULIACEÆ.

STERCULIA PHILIPPINENSIS Merrill, new name (*Sterculia cordifolia* Blanco. Fl. Filip. ed. 1, 764; ed. 2, 525; ed. 3, 3:163, non Cav.; *Sterculia urens* F.-Vill., Nov. App. 26. 1880, non Roxb.).

This species is very characteristic, and is not closely related to *Sterculia urens* Roxb., to which it was reduced by F.-Villar. The latter species does not extend to the Philippines. Blanco's name is preoccupied by that of Cavanilles, and accordingly the name given above is here proposed. *Sterculia philippinensis* is well represented by No. 2159, Merrill, Pinamalayan, Mindoro, May, 1903, and No. 225, Forestry Bureau, collected by Gammill, Guimaras Island, January, 1904, both specimens in fruit. V., *Banilad*.

MALVACEÆ.

ABELMOSCHUS SHARPEI Copeland, sp. nov.; perennial, with woody base, all younger parts hispid; leaves with sagittate base, 3 to 5 partite, segments linear, entire or nearly so; peduncles exceeding petioles of their subtending leaves; bracteoles 7 to 10, linear, shorter than calyx.

Davao, Mindanao, in wet pastures, Copeland No. 364.

Usually branched from the base, 40 to 100 cm. high; pedicels and peduncles 2 to 4 cm.; longest lobes of leaves 7 cm. long, 5 mm. wide; bracteoles 10 to 12 mm.; calyx 3 to 5 toothed; petals 2.5 to 4 cm. long, yellow, reddish toward base, turning maroon; capsule 2 to 2.5 cm. long, globose-oblong; seeds reniform, not musky.

This plant seems to be as near *A. moschatus* Moench, as to any species, but differs in the woody base, smaller flowers, narrow entire leaf lobes, short capsules, and nonmusky seeds. *A. moschatus* has been collected in Paragua by Merrill (795), and is probably widespread; but specimens from near Manila determined as such by J. Perkins are remarkably distinct in their bracteoles.

GUTTIFEREÆ.

KAYEA PANICULATA (Blanco). (*Plinia paniculata* Blanco, Fl. Filip. ed. 1, 423; ed. 2, 296; ed. 3, 2:184; *Kayea racemosa* F. Villar, Nov. App. 17. 1880, non Pl. et Tr.)

A medium-sized tree with oblong-lanceolate, acuminate leaves, the inflorescence in axillary and terminal panicles and racemes, 5 to 7 cm. long. Branchlets, slender, gray, glabrous. Leaves thin, glabrous, shining, the base acute, the apex acuminate, 10 to 14 cm. long, 2.5 to 3.2 cm. wide, the nerves obscure, very numerous, the more prominent ones about 4 mm. apart; petioles 8 to 10 cm. long, rugose. Inflorescence few, or rather many flowered, the bracts, if present, early deciduous, the few branches opposite,

2 cm. long or less, each with from one to three flowers. Flowers white, 2 cm. in diameter. Sepals glabrous, orbicular, rounded, 3.5 to 4 mm. long, the outer two slightly thickened and rugose. Petals ovate, obtuse, 10 mm. long, 6 mm. wide. Fruit (immature) 1 cm. in diameter, the persistent, much-thickened accrescent calyx lobes covered with small brownish scales.

This species is rather common in the hill forests of Bataan, and is represented by the following specimens: Lamao, Province of Bataan, Luzon, No. 364, Forestry Bureau, collected by P. T. Barnes, March, 1904 (flower); No. 2539, Merrill, same locality (fruit), June, 1903.

These specimens are taken from the neighborhood where Blanco secured his type material, and agree perfectly with his description. According to Blanco the Tagalog name for this species in Bataan was *Guisian*; according to Barnes it is *Carinas*.

LECYTHIDACEÆ.

PLANCHONIA SPECTABILIS Merrill, sp. nov. (*Planchonia valida* Vidal, Sinopsis, Atlas, t. 50 f. D. non Blume.)

A large tree, reaching a height of 30 m. or more, entirely glabrous, with thin membranaceous, broadly ovate or obovate acuminate leaves and short erect racemes bearing solitary or few large showy flowers. Young branches slender, brown, glabrous. Leaves alternate 10 to 15 cm. long, 7 to 10 cm. wide, entirely glabrous, the margins finely serrate, the apex very sharply and abruptly acuminate, the acumen about 1 cm. long, the base cuncate, somewhat decurrent; nerves 11 or 12 pairs, obscure above, distinct beneath; petioles 1.5 to 2 cm. long, narrowly wing-margined. Racemes terminal erect, 1 to 3 flowered, 1 cm. long. Flower 7 cm. long, slightly fragrant. Calyx tube funnel shaped, cylindrical or very obscurely angled, glabrous, 1 cm. long, the lobes four, imbricate round-ovate, 5 mm. long, 8 to 10 mm. wide. Petals obovate-oblong, pale green, acute or obtuse, 3 cm. long, 12 to 13 mm. wide. Staminal tube nearly 2 cm. long, the stamens 6 cm. long, the filaments slender, white at the apex, shading to deep red at the base; anthers yellow. Style, stigma and ovary green, the former very slender, 6 cm. long. Fruit ovoid, glabrous, green, not compressed or angled, 4.5 cm. long, 3 cm. thick, the calyx crowned apex 1 cm. in diameter. Seeds irregularly compressed, few, 4 to 6, 1.5 cm. long.

Type specimen No. 58 Forestry Bureau, collected by Mr. P. T. Barnes at Lamao River, Province of Bataan, Luzon, October, 1903; No. 2612, Merrill, from the Province of Tayabas, Luzon, appears also to be a form of this species.

This tree reaches a large size and grows in the hill forests up to an elevation of 600 m., where it was observed by the author in October, 1903. The type was collected by Mr. Barnes at an elevation of about 100 m. The trunk is straight and columnar, reaching a diameter of 1 m., the bark being brown and very scaly. It is not uncommon in the forests at Lamao River, but so far as known is of no economic value. The flowers are produced in great abundance, but are of short duration, falling in the early morning, often hundreds of fresh-fallen flowers being found under a tree. T., Lamaog; V., Uban.

COMBRETACEÆ.

TERMINALIA.

The first Philippine species of *Terminalia* described are *T. latifolia* Blanco, and *T. angustifolia* Blanco, in the first edition of his *Flora de Filipinas* in 1837. In the second edition of the same work, published in 1845, the former was changed to *Terminalia mauritiana*, and the latter to *Terminalia edulis*. *Terminalia latifolia* Blanco and *T. mauritiana* Blanco, are synonyms of *Terminalia catappa* L. *T. edulis* Blanco, is a valid species, *T. angustifolia* Blanco, being a synonym. In 1845 Blanco described a third species of *Terminalia* under the generic name *Gimbernatia*, this species, *G. calamansanai*, not being transferred to *Terminalia* until 1884. In 1849 Presl described in his *Epimiliæ Botaniciæ*, based on Cuming's Philippine material, four species of *Terminalia*—*T. polyantha*, *T. parviflora*, *T. nitens*, and *T. pellucida*—and a fifth species sub *Pentaptera*, *P. mollis*. Of these species described by Presl, four are apparently valid, *Terminalia polyantha* here being reduced to *Terminalia catappa* L. The next consideration of Philippine *Terminalia* is by Fernandez-Villar, in the *Novissima Appendix* to the third edition of the *Flora de Filipinas*, in 1880. F.-Villar enumerates six species with numerous synonyms, nearly the entire list being erroneous, and at the same time but one of the five species described from Philippine material by Presl, is mentioned and that as a synonym. Of the list of *Terminalia* given by F.-Villar, *Terminalia catappa* with synonyms is correct, *Terminalia belerica* F.-Vill., non Roxb., var. *typica* is *Terminalia edulis* Blanco. The variety No. 2 of F.-Villar is unknown, but should be excluded. *Terminalis belerica* var. *laurinoides* F.-Vill., non T. et B., is *Terminalia nitens* Presl., *T. procera* F.-Vill., non Roxb., is *Terminalia magarapali* Vidal, *Terminalia chebula* Retz., is credited to the Philippines, but this is undoubtedly an error, as F.-Villar reduces to this species *Bucida comintana* Blanco, but it is at once evident from Blanco's description, that *Bucida comintana* is not a species of *Terminalia*, but probably *Calycopteris*. *Terminalia arjuna* Bedd., is another species undoubtedly erroneously credited to the Philippines by F.-Villar, no synonyms are given, and it is impossible to determine at this time just what the species may be, so referred by F.-Villar. *Terminalia bialata* F.-Vill., non Kurz, is *Terminalia calamansanai* (Blanco) Rolfe.

In 1883 Vidal's "Sinopsis" was published, and in the *Atlas* of this work three species of *Terminalia* are partially figured. Of these three species, *Terminalia belerica* Vidal, non Roxb., is *Terminalia edulis* Blanco, *Terminalia bialata*, Vidal, non Kurz, is *Terminalia calamansanai* (Blanco) Rolfe, *Terminalia magarapali* Vidal, is here first described, but the description is very imperfect and only the fruit is figured. This species is, however, most distinct, but as yet has not been collected a second time. In 1884 Rolfe transferred to *Terminalia*, *Pentaptera mollis* Presl, and *Gimbernatia calamansanai* Blanco. The last enumeration of Philippine *Terminalia* is the list given by Vidal in his *Revision*, published in 1886, where nine species

are noted, *Terminalia magarapali* Vidal, is not listed here, from which it is evident that the type of this species was lost or destroyed before 1886. In the present enumeration eleven species of *Terminalia* are discussed, of which three are proposed as new.

Key to the Philippine species of Terminalia.

Leaves ovate, lanceolate or elliptical.

Leaves obtuse, glabrous; flower glabrous on the outside;
fruit obovate two or three winged..... *T. parviflora*

Leaves acuminate or acute.

Inflorescence paniculate; flowers glabrous on the
outside..... *T. multiflora*

Inflorescence spicate; flowers densely pubescent on
the outside.

Fruit with broad, thin wings *T. calamansanai*

Fruit ovate, compressed, wingless..... *T. edulis*

Leaves obovate.

Leaves pilose..... *T. mollis*

Leaves glabrous or nearly so.

Fruit not compressed or keeled.

Fruit ellipsoid, 4 to 4.5 cm. long, 3 to 3.5 in diam-
eter, rounded at both ends; leaves 5.5 to 7
cm. wide *T. ellipsoidea*

Fruit egg-shaped, 4.5 to 5.5 cm. long, 3 to 3.5
cm. in diameter; leaves 8 to 10 cm. wide --- *T. ovocarpa*

Fruit 4 cm. long, 1.5 cm. in diameter, the apex
acuminate; leaves brown, glossy *T. nitens*

Fruit 2 to 2.5 cm. long *T. pellucida*

Fruit more or less compressed, keeled.

Fruit less than 4 cm. long, strongly keeled.... *T. catappa*

Fruit often 10 cm. long, slightly compressed and
keeled; leaves truncate or with a short
acumen, the margins undulate; petioles 2 cm.

long, with two glands at the apex *T. magarapali*

TERMINALIA OVOCARPA Merrill, sp. nov.

A large tree with glabrous, obovate leaves and egg-shaped fruits 4.5 to 5.5 cm. long. Ultimate branches somewhat thickened, glabrous, the older branchlets marked with many prominent leaf scars. Leaves 13 to 15 cm. long, 8 to 10 cm. wide, glabrous on both surfaces, minutely pellucid-punctate, tapering from above the middle to the cuneate base, the apex very abruptly acuminate, or rarely rounded or emarginate, the acumen blunt, obscurely emarginate, less than 5 mm. long; nerves 10 pairs, rather prominent beneath, anastomosing near the margins; petioles glabrous, 2 cm. long, with two glands near the middle. Flowers unknown. Fruit egg shaped, 4.5 to 5.5 cm. long, 3 to 3.5 cm. in diameter, the base rounded, the apex subacute, pericarp 2 to 6 mm. thick, spongy, the endocarp hard.

Type specimen No. 67, Forestry Bureau, collected by P. T. Barnes at Lamao River, Province of Bataan, Luzon, November, 1903.

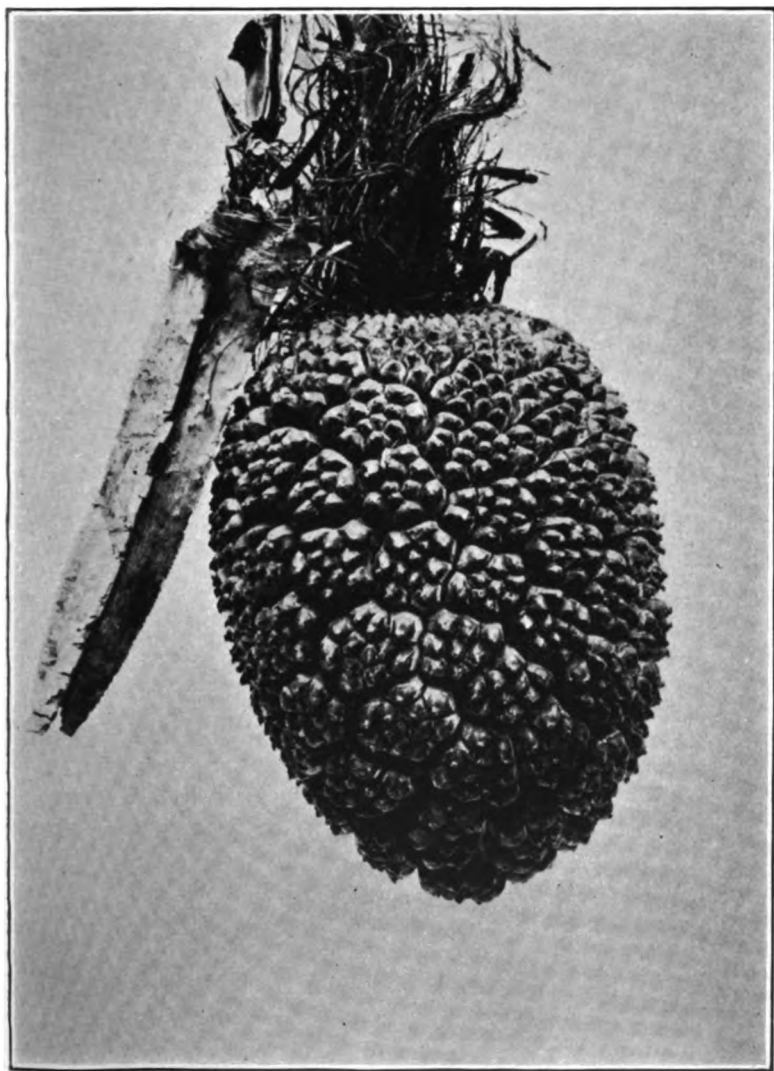


PLATE III. *PANDANUS ARAYATENSIS* MERRILL, FRUIT.

A large tree, growing in dry hill forests at an elevation of 100 m. above the sea, with columnar, buttressed trunks nearly 1 m. in diameter and about 25 m. to the first branches, the buttresses nearly 1 m. wide at the base, extending to a height of 2 m. Bark very scaly, light gray. The wood is brown and is used by the natives in the construction of bancas (native canoes) and for boards in the construction of houses. Locally known to the Tagalogs of Bataan as "*Talisay del monte*."

This species is distinguished from *Terminalia pellucida* Presl, by its much larger fruits, broader and entirely glabrous leaves, and glabrous petioles; from *Terminalia magarapali* Vidal, in its smaller, differently shaped and not angled fruits, and from *T. phellocarpa* King, a species of the Malayan Peninsula, especially in its much smaller, narrower leaves.

TERMINALIA ELLIPSOIDEA Merrill, sp. nov.

A tree with glabrous, obovate, abruptly blunt-acuminate leaves, the nerves of which are prominent beneath, and ellipsoid fruits 4 to 4.5 cm. long. Ultimate branches somewhat thickened, rusty pubescent, becoming glabrous. Leaves subcoriaceous 10 to 14 cm. long, 5.5 to 7 cm. wide, the under surface minutely and obscurely punctate, and with few rusty hairs along the midnerve, the margins slightly undulate, apex, except for the abrupt blunt acumen, rounded, the base cuneate; nerves 11 pairs, prominent beneath; petioles 1 to 1.5 cm. long, appressed rusty-pubescent when young and with two glands at the upper third. Flowers unknown. Fruit ellipsoid glabrous, not compressed, rounded at both ends, 4 to 4.5 cm. long, 3 to 3.5 cm. thick.

Type specimen No. 2148 Merrill, Pinamalayan, Mindoro, May, 1903. Locally known to the Tagalogs as *Calumpit*, but this name is usually applied to *Terminalia edulis* Blanco.

A tree 18 m. high, and 40 to 50 cm. in diameter with rough grayish-red bark, and ash-colored wood, which is used by the natives in the construction of houses and boats.

TERMINALIA NITENS Presl, Epim. Bot. 213. 1849; Walp. Ann. 3:859. 1853; Vidal, Rev. Pl. Vasc. Filip. 127. 1886; *Terminalia belerica* Roxb. var. *laurinoides* F.-Vill., Nov. App. 80. 1880, non *Terminalia laurinoides* T. et B.

Type locality: "Habitat in insula Luzon." (No. 1326 Cuming.) Specimens examined: Luzon, Province of Bataan, Lamao River, No. 64, Forestry Bureau, collected by P. T. Barnes; Province of Rizal, Bosoboso, No. 2800, Merrill. T., *Sacat*. A species not as yet found outside of the Philippines, although *Terminalia phellocarpa* King, from the Malayan Peninsula, is closely related.

TERMINALIA EDULIS Blanco, Fl. Filip. ed. 2, 265. 1845, l. c. ed. 3, 2:127; Vidal, Rev. Pl. Vasc. Filip. 127. 1886. *Terminalia angustifolia* Blanco, Fl. Filip. ed. 1, 377. 1837, non Jacq. *T. belerica* F.-Vill., Nov. App. 80. 1880; Vidal, Sinopsis, Atlas, t. 48. f. B. non Roxb.

Specimens examined: Luzon, Province of Rizal, Antipolo, No. 1626, Merrill; Tanay, No. 2283, Merrill; Province of Zambales, No. 2908, Merrill;

Province of Bataan, Balanga, No. 306, Ahern; Province of Tayabas, Lagui-manoc, No. 2588, Merrill; Malicboi, No. 35, Ritchie; Province of Camarines, Dalupaon, No. 1, Ahern; Island of Masbate, No. 2759, Merrill.

This common endemic species is universally known to the Tagalogs as *Calumpit*. Other names, Zambales, *Gayumayen*; V., *Magtalisay*, *Calumanog*; (Masbate), *Cotmoc* (Camarines).

TERMINALIA MULTIFLORA Merrill, sp. nov.

A large tree with paniceled terminal inflorescence and glabrous ovate or lanceolate leaves, which are not crowded at the apices of the branches. Branches dark brown, glabrous, subopposite. Leaves alternate or subopposite, 5 to 8 cm. long, 2.5 to 3.5 cm. wide, the base rounded or subacute, the apex acuminate or acute; nerves 11 or 12 pairs, spreading, curving upwards, the upper surface minutely white punctate, the lower surface finely brown-reticulate, glabrous, the petioles 1.5 cm. long, with two glands at the apex. Panicles much branched, the spike-like ultimate branches many flowered 5 to 9 cm. long, 6 to 8 cm. wide, the axis and branches densely brown tomentose. Flowers all perfect, sessile, 4 mm. long, including the exerted stamens, 2 to 2.5 mm. wide, the younger ones subtended by a linear-lanceolate, deciduous bracteole, 1.5 mm. long, slightly hirsute at the base, otherwise glabrous; calyx glabrous on the outside, the spreading border densely villous inside, the teeth acute. Ovary glabrous, oblong, 1 mm.; stamens, 10, the filaments glabrous, 2.5 long; style glabrous, equaling the stamens.

Type specimen No. 2796, Merrill, Bosoboso, Province of Rizal, Luzon, July, 1903, known to the Tagalogs of Rizal as *Naghubo*; No. 2647, Merrill, from the same locality, with very young fruit, is also referred here.

A species evidently related to *Terminalia chebula* Retz, but abundantly distinct, so far as can be determined from the descriptions of that species available. This species may represent the form credited to the Philippines by F.-Villar¹ as *Terminalia chebula* Retz., but it is impossible to determine this as F.-Villar gives no description and no specimens were preserved.

F.-Villar² without comment refers Blanco's *Bucida comintana* to the species he considered to represent *Terminalia chebula*, without having seen authentic specimens of either species. A cursory examination of Blanco's description shows at once that *Bucida comintana* can not be referred to *Terminalia*, as the fruit is described as being crowned by the persistent calyx. Blanco's *Bucida comintana* is undoubtedly a species of *Calycopteris*, but judging from his description is distinct from *Calycopteris floribunda* Lam.

TERMINALIA MOLLIS (Presl) Rolfe, Journ. Linn. Soc. Bot. 21:310. 1884; Vidal, Rev. Pl. Vasc. Filip. 127. 1886; *Pentaptera mollis* Presl, Epim. Bot. 214. 1849; Walp. Ann. 3:859. 1853.

Type locality: "Habitat in insula Luzon" (No. 1004 Cuming).

This species is represented by No. 6395 A. D. E. Elmer, Twin Peaks, Province of Benguet, Luzon, June, 1904.

¹ Nov. App., Fl. Filip., 80.

² l. c.

TERMINALIA PELLUCIDA Presl, Epim. Bot. 214. 1849; Walp. Ann. 3:859. 1853; Vidal, Rev. Pl. Vasc. Filip. 127. 1886.

Type locality "Habitat in insula Luzon." (No. 1326 Cuming.) Specimens examined: Luzon, Province of Tayabas, Pagbilao, No. 1943, 1951, 2846, Merrill; Province of Rizal, Bosoboso, No. 1829, Merrill. These specimens do not agree entirely with Presl's description, but are so referred. Nos. 1755 and 2901, Merrill, from the Province of Zambales, appear to well represent the species.

TERMINALIA MAGARAPALI Vidal, Synopsis, Atlas, XXVI. t. 48. f. c. 1883. (*T. procera* F.-Vill. Nov. App. 80. 1880, non Roxb.)

This species is not listed in Index Kewensis, and is based on a specimen collected on the Island of Alabat, off the Pacific coast of Tayabas Province, Luzon. The type has undoubtedly been destroyed, as this species is not mentioned by Vidal in his "Revision de Plantas Vasculares Filipinas" in 1886. It is characterized by its large, obovate glabrous leaves, and very large, somewhat compressed and keeled fruits, which are often 10 cm. long. It is known only from Vidal's imperfect description and figure of the fruit. The Tagalogs of Alabat Island know this species as *Magarapali*.

TERMINALIA CATAPPA Linn. Mant. 2:519. 1771; Hook; Fl. Brit. Ind. 2:444; Vidal, Rev. Pl. Vasc. Filip. 127. 1886. *T. latifolia* Blanco, Fl. Filip. ed. 1, 376. 1837, non Swartz. *T. mauritiana* Blanco, l. c. ed. 2, 264. 1845, non Lam. *T. polyantha* Presl, Epim. Bot. 213. 1849. ex. descr.

This species is found throughout the Malayan region, and is very common and widely distributed along the coast in the Philippines, being much cultivated for shade, but proving very unsatisfactory for this purpose in Manila. There is no doubt as to the proper reference of Blanco's names. Presl's *Terminalia polyantha* was based on No. 1516 Cuming, from Mindoro, and judging from his description is only a form of *Terminalia catappa* L., although it is held distinct by Vidal¹. This species is universally known in the Philippines as *Talisay*, while many of the Spanish-speaking people call it *Almendras*.

TERMINALIA CALAMANSANAI (Blanco) Rolfe, Journ. Linn. Soc. Bot. 21: 310. 1884; Vidal, Rev. Pl. Vasc. Filip. 127. 1886. *Gimbernatia calamansanai* Blanco, Fl. Filip. ed. 2, 266. 1845. *Terminalia bialata* F.-Vill. Nov. App. 80. 1880; Vidal, Synopsis, Atlas, t. 48. f. B. 1883, non Kurz.

Specimens examined: Luzon, Province of Zambales, Botolan, No. 2981, Merrill; Province of Principe, Baler, No. 1069, Merrill. Mindanao, Province of Surigao, No. 329, Ahern.

A common and widely distributed endemic species, known to the Tagalogs as *Calamansanai*, and *Malacalumpit*, and to the Visayans as *Lumanog*.

Blanco has included two forms in this species, which may prove to be distinct when more material is available for comparison. One form mentioned has fruits 1½ inches wide, and the other has fruits 2½ inches wide.

¹ Phan. Cuming. Philip. 112.

TERMINALIA PARVIFLORA Presl, Epim. Bot. 214. 1849; Walp. Ann. 3:858. 1853.

Type locality "Habitat in insula Luzon." (Cuming 1439, Batangas.) This endemic species is characterized by its ovate-elliptical, obtuse leaves, and obovate, two to three winged fruits. It has been collected by Lohrer, and is represented in our herbarium by No. 785 Ahern, Mariveles, Province of Bataan, Luzon. T., *Calamansanai*.

A species evidently closely related to *Terminalia calamansanai* Rolfe.

MYRTACEÆ.

JAMBOSA BATAANENSIS Merrill, sp. nov.

A small tree, 8 m. high or less, with lanceolate, acuminate, few-nerved leaves, and large, solitary flowers which either terminate the branchlets, or are borne on the larger branches, and rarely also on the trunk of the tree. Branches gray, smooth, the branchlets brownish, slender, terete, glabrous. Leaves glabrous, 7 to 10 cm. long, 2 to 3 cm. wide, the apex with a rather slender, elongated, but blunt acumen, the base acute, rarely somewhat obtuse, the upper surface dark colored, the lower pale; nerves very irregular, about 10 on each side of the midrib, not prominent, reticulations lax; marginal nerves two, the outer very faint; petioles less than 2 mm. long. Flowers 3 cm. long, 4 to 5 cm. wide, when open, red and white, or pinkish, the peduncle slender 1 to 1.5 cm. long. Calyx 1.5 cm. long, 1.5 cm. wide, funnel shaped, the lobes four, irregular, 4 to 6 mm. long, 8 to 10 mm. wide. Petals orbicular, distinct, four, 1 cm. in diameter, the base pink, the tip red. Filaments 2 to 2.5 cm. long, the base deep red, the tip white or pink; anthers 1.5 mm. long. Style about 4 cm. long.

Type specimen No. 3761, Merrill, Mount Mariveles, Province of Bataan, Luzon, January 1, 1904. Rather common on forested ridges at elevations of from 800 to 1,000 m. above the sea.

JAMBOSA GARCIE Merrill, sp. nov.

A tree with very large sessile, ovate-lanceolate or oblong leaves, strongly four-angled branches and terminal panicle inflorescence. Branchlets brown, glabrous, about 1 cm. in diameter, strongly four-angled, the angles winged. Leaves coriaceous, glabrous, about 40 cm. long, 15 cm. wide, the apex acute or slightly acuminate, the base rather abruptly narrowed, rounded, strongly cordate, the upper surface dark brown, the lower pale when dry, the midnerve very prominent, brown; primary nerves prominent, about 20 on each side of the midrib, 1 to 2 or 3 cm. apart, the reticulations lax, but rather prominent, the main nerves arching and anastomosing near the margin. Panicles 20 cm. long or more, the axis and stout spreading branches and branchlets four-angled, the lower branches 8 cm. long; bracts ovate, 3 to 4 mm. long. Flowers sessile, 1 cm. long, 1.5 cm. wide when open, each subtended by a whorl of four small bractlets. Calyx turbinate, about 5 mm. in diameter, four lobed, the lobes 1.5 mm. long, 3 mm. wide. Petals orbicular, 5 to 6 mm. in diameter, thick, coriaceous. Filaments about 1 cm. long; anthers 0.5 mm. long. Style 1 cm. long.

Type specimen collected at Pola, Mindoro, by R. Garcia, an employee of

the Forestry Bureau, May, 1903, distributed as No. 2367, Merrill, under the name *Eugenia garciae* Merrill.

JAMBOSA LONGIPEDICELLATA Merrill, sp. nov.

A small tree with broadly ovate, obtuse, strongly cordate sessile leaves, and much elongated, three-flowered, racemose inflorescence. Branches terete, glabrous, light gray. Leaves glabrous, coriaceous, 12 to 14 cm. long, 6.5 to 8 cm. wide, scarcely narrowed at the abruptly rounded, strongly cordate base; primary nerves 8 to 10 on each side of the midrib, with one or two less prominent secondary nerves between each two primary nerves, the primary nerves anastomosing 8 to 10 mm. from the margin, reticulations lax, rather prominent. Inflorescence terminal, solitary, or two peduncles from the tip of the same branchlet, the peduncle very much elongated, slender, 20 to 25 cm. long, the pedicels 5 cm. long. Flowers about 3 mm. long, and 4 mm. wide or more when open, the buds obovate. Calyx funnel-shaped, the tube 1 cm. long, 1.5 cm. in diameter, four lobed, the lobes large, very unequal, one pair 1.5 cm. wide, 9 to 12 mm. long, the other 8 to 10 mm. wide, 4 to 8 mm. long. Petals 2.5 cm. long, 1.5 cm. wide. Filaments 2 cm. long; anthers 2 mm. long. Style 3 cm. long.

Type specimen No. 1046, Merrill, Baler, Province of Principe, Luzon, August, 1902. T., *Lipote*.

JAMBOSA BARNESII Merrill, sp. nov. § *Eujambosa*.

A tree 10 to 15 m. high, with glabrous, ovate or ovate-lanceolate, few-nerved, acuminate leaves, which are cordate at the base, and terminal panicles of medium-sized white flowers. Branches slender, glabrous, light gray, terete or the ultimate branchlets sometimes four angled. Leaves firm, plumbeous when dry, glabrous, the upper surface shining, the lower surface with small scattered glands, dull, 8 to 13 cm. long, 3.5 to 5.5 cm. wide, the apex rather abruptly acuminate, rounded below to the slightly cordate base; main nerves about 13 on each side, very irregular, anastomosing in a subprominent marginal nerve at a distance of about 4 mm. from the margin, between this nerve and the edge of the leaf is a second one very faint, reticulations lax, not prominent; petiole dark colored 2 mm. long. Panicles 8 to 10 cm. long, about the same diameter, terminal, the branches ascending, the lower ones 5 to 6 cm. long. Flowers 2 cm. long, 2 cm. wide when open, white, fragrant, the flower buds globose. Calyx funnel shaped, 1 cm. long, nearly truncate, or with inconspicuous rounded lobes. Petals 5, orbicular, 5 mm. in diameter. Filaments 12 mm. long. Style 10 mm. long or more.

Type specimen No. 140, Forestry Bureau, collected by P. T. Barnes, Lanao River, Mount Mariveles, Province of Bataan, Luzon, January, 1904.

A small tree reaching a diameter of 15 cm., growing in dry hill forests at an elevation of 800 m. above the sea.

JAMBOSA LUZONENSIS Merrill, sp. nov.

A tree 10 m. high, with glabrous, lanceolate, acuminate, few-nerved leaves, which are acute at the base, and axillary few-flowered panicles of medium-sized white flowers. Branchlets small, glabrous, terete, brownish. Leaves 9 to 14 cm. long, 3.5 to 5 cm. wide, the base acute, the apex acumi-

nate; main nerves 8 or 9 on each side of the midrib, distant, anastomosing 3 to 7 mm. from the margin, a second indistinct marginal nerve between the nerve thus formed and the margin, reticulations fine, obscure; petioles thickened, rugose, dark colored, 1 cm. long. Inflorescence from the branches and branchlets below the leaves, about 8 cm. long, the few branches spreading or ascending, each bearing two to four flowers. Flowers about 1.5 cm. long, 2 cm. wide when open, slightly fragrant, the buds obovate, the pedicels about 4 mm. long. Calyx turbinate, 7 mm. long, 5 mm. wide, four lobed, the lobes orbicular, very unequal, two about 4 mm. long and 5 mm. wide, the other two about 3 mm. in diameter. Petals orbicular, 6 to 7 mm. in diameter, distinct, rather densely punctate with prominent but small yellow glands. Filaments 1.5 to 2 cm. long; anthers 1 mm. long. Style about 1.5 cm. long.

Type specimen No. 83, Forestry Bureau, collected by P. T. Barnes, Lamao River, Province of Bataan, Luzon, November, 1903.

A tree with yellowish-red bark, growing in the forests along the river at an elevation of 100 m. above the sea. T., *Malaruhat*, but this name is applied to various other species of *Jambosa*.

Jambosa luzonensis is apparently closely related to the species described by Blanco as *Myrtus subrubens*, which is a true *Jambosa*, but Blanco's description does not apply to the species here described.

SYZYGium pallidum Merrill, sp. nov.

A small tree with lanceolate, acuminate, glabrous, very pale leaves, and terminal and axillary panicles of small flowers. Branchlets terete, glabrous, slender, brownish gray. Leaves 5 to 7 cm. long, 1.5 to 3 cm. wide, coriaceous, with very numerous inconspicuous nerves, pale above, very pale, almost white, beneath, the apex and base acuminate, or the latter sometimes acute; petioles about 1 cm. long. Panicles many flowered, 4 to 6 cm. long, the lower branches ascending, about 2 cm. long. Flowers 6 to 7 mm. long, including the stamens. Calyx crateriform, 4 mm. long, truncate, 2 mm. in diameter. Corolla calyptrate, falling as a whole. Filaments 4 to 5 mm. long; anthers 0.4 mm. long. Style about 4 mm. long.

Type specimen No. 1764, Merrill, Subig, Province of Zambales, Luzon, April, 1903.

A very characteristic species, recognized by its pale greenish-white leaves (when dry).

MELASTOMACEÆ.

MELASTOMA TOPPINGII Merrill, sp. nov.

A shrub with narrowly ovate, acute, 5-nerved leaves, angular ultimate branchlets, which are densely clothed with long brownish or reddish hairs, and lanceolate-acuminate calyx lobes shorter than the calyx tube. Branches brownish, the clothing hairs of the ultimate branchlets minutely scabrid, 2 to 4 mm. long, more or less spreading and curved upward. Leaves 5 to 8 cm. long, 2 to 3.5 cm. wide, the base slightly rounded, the upper surface rather densely clothed with short, stiff, subappressed hairs, beneath rather densely strigose-pubescent, the hairs on the nerves beneath

longer and more appressed; petioles densely clothed with long hairs, 5 to 8 mm. long. Flowers purplish, fasciculate, usually in threes. Calyx tube 1 cm. long, densely clothed with more or less appressed stout hairs 1 to 3 mm. long, which are arranged in fascicles, the calyx lobes lanceolate-acuminate, 7 mm. long, 2 mm. wide, near the base densely ciliate with long hairs, the alternating teeth slender, 2 mm. long, ciliate, and with a tuft of long, penicillate hairs at the apex. Petals obovate, 2 to 2.3 cm. long, 1.5 cm. wide, the margins ciliate. Larger stamens 2 cm. long, the anther 9 mm. long, the connective scarcely elongated, 1.5 mm. or less in length.

Type specimen No. 17, D. LeRoy Topping, Baguio, Province of Benguet, Luzon, January, 1903.

MELASTOMA FUSCA Merrill, sp. nov.

A shrub 2 m. high or less, with lanceolate, acuminate, 5-nerved leaves, the lobes of the calyx broad, nearly truncate, one-half as long as the calyx tube. Branches pale brown, densely clothed with appressed, brownish, lanceolate, chaffy scales, 1 mm. long or less. Leaves 9 to 13 cm. long, 2 to 3.5 cm. wide, the base acute, the apex acuminate, rarely acute, the marginal nerves not prominent, both surfaces scabrous with short, scattered, subappressed, stiff hairs; petiole pale brown, scaly, 1 to 1.5 cm. long. Flowers purplish, solitary in the axils of the leaves or three or four together at the ends of the branches, short pedicellate. Bracts obovate, 2 cm. long, 1.5 cm. wide, densely scaly, the margins ciliate. Calyx tube pale brown, 10 to 12 mm. long, densely clothed with imbricated lanceolate, ciliate, appressed scales 2 mm. long or less, the lobes 6 to 7 mm. long, 4 to 5 mm. wide, the apex very abruptly apiculate, almost truncate, densely scaly, the alternating teeth very short, less than 1 mm., penicillate-ciliate. Petals obovate, 2.5 cm. long, 1.7 cm. wide. Longer stamens 3 cm. long, the anther 12 mm. long, the connective 10 mm. long.

Type specimen No. 340, Forestry Bureau, collected by P. T. Barnes, Lamao River, Province of Bataan, Luzon, February, 1904. No. 204 Forestry Bureau, collected by Barnes in the same locality, January, 1904, is the same.

ASCLEPIADACEÆ.

DISCHIDIA PURPUREA Merrill, sp. nov.

An epiphytic, herbaceous, creeping vine on erect tree trunks, rooting at every node, the fleshy coriaceous leaves flattened against the supporting tree trunk, the hollow space formed by the shield-like leaves being occupied by colonies of ants, a single much-branched root, from each axil spreading over the entire area covered by the leaf. Stems slender, glabrous, sometimes 1 m. long, the internodes 2 to 4 cm. long. Leaves in pairs, opposite, sessile, orbicular, fleshy, coriaceous deep purple, glabrous when fresh, when dry the under surface minutely warty, 3.5 to 5 cm. in diameter, nerves 4 or 5 pairs, rather prominent beneath, obsolete above. Inflorescence axillary, the peduncle slender, 4 cm. long, the apex with two short divergent much-thickened branches, the flowers produced from the tips of the branches. Pedicels glabrous, slender, 1.5 mm. long. Flowers few, 4 to 4.5 mm. long,

pink to white, glabrous throughout. Sepals thin, ovate, obtuse or subacute, 1 mm. long. Corolla urceolate, membranous, 3 mm. in diameter, 4 mm. long, the lobes triangular, acute, 1 mm. long. Cbronal scales, five, the tips 2-fid, recurved, 0.5 mm. long. Column 2 to 2.5 mm. long.

Type specimen No. 3735, Merrill, growing on tree trunks on exposed wind-swept ridges, Mount Mariveles, Province of Bataan, Luzon, January 1, 1904, at an elevation of 1,200 m.

This species of the section *Conchophyllum* is distinguished from the widely distributed *Dischidia imbricata* K. Sch., by its larger flowers, which are not subsessile, and rather larger leaves, which have more numerous veins than the leaves of *Dischidia imbricata*.

There are several distinct species of the genus *Dischidia*, section *Conchophyllum*, common and widely distributed in the Philippines, but the various ones are rarely found in flower, and accordingly can not be readily identified. The genus is especially interesting on account of the symbiosis with ants, colonies of these insects being almost invariably found living under the shield-like leaves of the species of the section *Conchophyllum*, and inside of the hollowed pendant leaves of that of the section *Ascidiphora*. The much-branched axillary roots which are found covering the space protected by the flattened leaves of the species of the former, and inside of the hollow leaves of the species of the latter, evidently serve the purpose of assimilating the refuse matter of the living ants, while the peculiar leaves in both cases conserve moisture for the plant.

CONVOLVULACEÆ.

RIVEA BARNESII Merrill, sp. nov.

A woody vine reaching a height of 30 m. and a diameter of 4 cm. with ovate-acuminate, coarsely papillate-pubescent leaves, and lavender flowers, the corolla very densely silky pilose on the outside with long appressed yellowish hairs, deeply lobed. Ultimate branches brownish, densely pubescent. Leaves 8 to 10 cm. long, 4 to 5.5 cm. wide, the base broad, rounded or subcordate, tapering from the lower third to the slender acuminate apex, the margins obscurely undulate, both surfaces uniformly and rather thickly covered with rather harsh upwardly curved white hairs, each hair from a small papilla; nerves about 7 pairs; petioles 2 to 2.5 cm. long, densely pubescent. Inflorescence cymose, from the axils of the leaves; peduncles pubescent, 3 to 4 cm. long, one to three flowered, pedicels thick 4 to 5 mm. long, pubescent. Bracts pubescent, lanceolate, 1 cm. long. Calyx 8 mm. long, the lobes rounded pubescent. Corolla 3 to 3.5 cm. long, the outside densely silky pubescent, deeply five lobed, the lobes lanceolate, 2 cm. long, 3 to 4 mm. wide, acute. Stamens five, alternating with the lobes, thickened below, and with a sharp inward curve above the base, closely surrounding the pistil, but not connivent, 2 cm. long, not exerted. Anthers 4 mm. long. Style slender glabrous, equaling the stamens; stigma two lobed, the lobes globose. Ovary two celled, two ovules in each cell. Fruit ovoid, 1 cm. long, one seeded.

Type specimen No. 68, Forestry Bureau, collected by P. T. Barnes, Lamao

River, Province of Bataan, Luzon, November, 1903, growing in dry hill forests, at an elevation of about 100 m. No. 1657 Merrill, Antipolo, Province of Rizal, Luzon, March, 1903, is the same.

SAPOTACEÆ.

CHRYSOPHYLLUM BOXBURGHII G. Don.

No. 2479, Merrill, Pola, Mindoro, June, 1903, represents this species, its previous known range having been from British India to Java and Sumatra. T., *Pisang dagá*.

Chrysophyllum grandifolium Steud., the only species of this genus previously reported from the Philippines, is very imperfectly known, having been described from leaf specimens only. Judging from the description it is a species of *Palaquium*, very close to, if not identical with, *Palaquium oleiferum* Blanco.

ILLIPE COBIACEA Merrill, sp. nov.

A tree with glabrous, coriaceous, oblong-obovate, obtuse leaves and ovate fruits. Branches glabrous, gray, the ultimate branchlets brown. Leaves brown when dry, 8 to 15 cm. long, 2.5 to 4 cm. wide, narrowed to the cuneate base; nerves obscure, 15 pairs; petiole about 2 cm. long, glabrous, rugose below, somewhat margined above by the slightly decurrent leaf blade. Flowers unknown. Fruit solitary, ovate, glabrous, 4 cm. long, 1.5 cm. broad, the peduncle 2 to 2.5 cm. long, glabrous, thickened above, the persistent calyx segments four, in two series, glabrous, rounded, 4 mm. long, the outer ones broader than the inner; seed solitary, 2.5 to 3 cm. long, ovate, acute, smooth, and shining, the hilum extending the length of the seed, 5 mm. broad; endosperm 2 cm. long, narrowly ovate, 8 mm. broad, the cotyledons 3 to 3.5 mm. thick.

Type specimen No. 1008, Merrill, Baler, Province of Principe, Luzon, August, 1902. T., *Lisong insic*.

ILLIPE MULTIFLORA Merrill, sp. nov.

A tree with ovate-lanceolate, coriaceous leaves which are at first minutely pubescent beneath, becoming glabrous, both surfaces shining, the flowers very numerous, crowded in fascicles of ten or more flowers each, in the axils of the leaves, or in the axils of fallen leaves, toward the ends of the branchlets. Ultimate branches reddish brown, thickened, the very tip rusty pubescent, otherwise glabrous. Leaves 11 to 16 cm. long, 4.5 to 8 cm. wide, the apex acute or slightly acuminate, below rounded to the generally somewhat acute, rarely obtuse base, the under surface densely and minutely silvery pubescent when young, becoming glabrous; nerves about fifteen pairs, subprominent on both surfaces, spreading, freely anastomosing near the margins; petioles glabrous, thickened and rugose below, 2 to 4.5 cm. long. Flowers greenish yellow, the pedicels 12 mm. long, densely rusty tomentose. Calyx 8 mm. long, densely rusty tomentose on the outside, the lobes broadly ovate, acute, 7 mm. long, 6 mm. wide, the two inner ones thinner than the outer, their margins glabrous. Corolla glabrous 9 to 10 mm. long, eight lobed, the lobes 7 mm. long, 3 mm. wide, the apex obtuse.

Stamens 20, the anthers sessile, 3.5 mm. long, clothed with long, stiff, white hairs. Ovary glabrous, nine celled. Fruit unknown.

Type specimen No. 411, Forestry Bureau, collected by Ahern's collector, Antipolo, Province of Rizal, Luzon, February, 1904; No. 762, Ahern, Mariaveles, Province of Bataan, Luzon, January, 1902, is the same. T., *Calamyanes*.

ILLIPE RAMIFLORA Merrill, sp. nov.

A tree reaching a height of 35 m., with oblong, glabrous, coriaceous leaves, five-parted calyx, the flowers borne on the medium-sized branches, not on the leaf-bearing branchlets. Branches grayish-brown, glabrous. Leaves 9 to 16 cm. long, 3.5 to 5 cm. wide, both surfaces smooth and shining, somewhat narrowed below to the abruptly acute or somewhat rounded base, the apex abruptly acute, the tip blunt; nerves obscure above, rather prominent below, ascending, 7 to 8 pairs, the reticulations lax, rather prominent, especially near the margins; petioles glabrous, thickened and rugose below, 1.5 to 3.5 cm. long, reddish brown. Flowers about 1 cm. in diameter, in fascicles of from three to six or more from protuberances on the branches, the pedicels 1.5 cm. long, appressed rusty-pubescent. Calyx five parted, the lobes very broadly ovate, 7.5 mm. long, 6 mm. wide, acute, coriaceous, densely pubescent with rusty appressed hairs. Corolla white, 8 mm. long, ten lobed, the lobes about 6 mm. long, 2 mm. wide, acute. Stamens 20; filaments 4 to 5 mm. long; anthers 4 mm. long. Ovary densely rusty pubescent, seven celled. Fruit unknown.

Type specimen No. 189, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, January, 1904; also a sterile specimen from the same locality, No. 583, Forestry Bureau, collected by Barnes, March, 1904.

A very characteristic species, which is perhaps worthy of being described under a new genus on account of its five-parted calyx, but as Engler includes such plants in the genus (*Illipe butyracea* (Roxb.) Engl.), it has been considered advisable to place the present species in the genus *Illipe*. A tree growing in the hill forests at an elevation of about 100 m., the latex produced being of no value. T., *Baniti*.

PAYENA LANCEOLATA Merrill, sp. nov.

A tree with glabrous lanceolate, acuminate leaves. Branches brownish gray, glabrous, the ultimate branchlets black when dry, deciduously rusty-pubescent. Leaves thinly coriaceous 8 to 11 cm. long, 3 to 4.5 cm. wide, brown when dry, entirely glabrous, the base acute, the apex acuminate; nerves 13 or 14 pairs, not prominent, dark brown beneath, anastomosing at the very margin; petioles 2 to 2.5 cm. long, glabrous or with very few rusty, appressed hairs; stipules caducous, 2 mm. long, ovate lanceolate, acute, densely pubescent with rusty appressed hairs. Inflorescence fasciculate in the axils of the branchlets or the fallen leaves, two to six flowers in a fascicle; peduncles 10 to 12 mm. long, dark brown or black when dry, pubescent with scattered appressed grayish hairs. Calyx lobes four, in two series, the outer broadly triangular-ovate, acute, 5 mm. long, 4 mm. wide, pubescent with scattered appressed grayish hairs, inner lobes thinner

than the outer, similar in shape and size, but slightly narrower, the exposed outer surface densely appressed pubescent, the margin with a narrow glabrous border, the edge sparingly ciliate. Corolla lobes (from immature flowers) 10, thin, glabrous, linear lanceolate, obtuse, 2.5 mm. long, 1 mm. wide. Stamens in two series, 18, the anthers lanceolate acuminate, 2 mm. long, clothed with very long (1 mm.) scattered appressed, rusty hairs. Fruit unknown.

Type specimen No. 493, G. P. Ahern, Island of Dinagat, 1901. Locally known as *Lonolono*.

This species in general appearance resembles *Payena lucida* A. DC., a species of the Malayan Peninsula, but differs from the latter in its longer petioles, more numerous corolla lobes, and stamens, the latter being glabrous in *Payena lucida*. *Payena lanceolata* should possibly be referred to the genus *Illipe*, but this point can only be determined by the examination of ripe seeds.

SIDEROXYLON RAMIFLOEBUM Merrill, sp. nov.

A tree with lanceolate or elliptic-lanceolate, nearly glabrous coriaceous leaves, the inflorescence in fascicles on simple specialized branches from above the scars of fallen leaves. Ultimate branches thick, densely brown tomentose, the older branches gray, glabrous, the leaf scars on the latter large and prominent, 4 to 5 mm. in diameter. Leaves 10 to 22 cm. long, 5 to 10 cm. wide, the base acute, the apex acute or acuminate, when young rather densely brown tomentose beneath, becoming glabrous in age, or the brown tomentum persistent along the nerves and midrib, glabrous above; nerves prominent, 11 pairs; petioles 3 to 5 cm. long, densely rusty tomentose when young, becoming glabrous. Branches bearing the inflorescence from above scars of fallen leaves, simple, rusty tomentose 2 to 8 cm. long, these branches rarely foliaceous at their apices, when foliaceous, often exceeding 8 cm. in length; fascicles 5 to 12 flowered; pedicels 5 to 6 mm. long, rusty tomentose; flower buds subglobose. Flowers 2.5 mm. long. Calyx rusty tomentose, the teeth acute, about 0.5 mm. long. Corolla 2 mm. long, the lobes obtuse, 1 mm. long, slightly less than 1 mm. wide, glabrous. Stamens not exerted; filament 0.5 mm. long, about equaling the nearly round anther. Connectives lanceolate-acuminate, about 8 mm. long. Ovary densely pubescent. Fruit unknown.

Type specimen No. 2793, Merrill, Bosoboso, Province of Rizal, Luzon, July, 1903; No. 3413, Merrill, from a specimen cultivated in the old Botanical Garden in Manila, October, 1903, and No. 77, Forestry Bureau, collected by P. T. Barnes at Lamao River, Province of Bataan, Luzon, are also referred here. This species is known to the Tagalogs of Rizal as *Bancalande*, and to those of Bataan as *Malapaho*, the latter name usually, however, being applied to a species of *Mangifera*.

This species is evidently most closely related to *Sideroxylon nitidum* Blume, from Java, but is distinguished by its leaf characters, acute calyx lobes, smaller flowers, etc.

PALAQIUM ANGUSTIFOLIUM Merrill, sp. nov.

A tree 8 to 10 m. high, with entirely glabrous, narrowly oblanceolate or

lanceolate leaves. Branches grayish brown, glabrous, the flower-bearing ones rough with many prominent leaf scars. Leaves 5 to 8 cm. long, 1.5 to 2.5 cm. wide, coriaceous, smooth and shining, the apex abruptly shortly blunt-acuminate, slightly or not at all tapering from the upper third to a point near the base, then rather abruptly cuneate; nerves prominent, 11 to 13 pairs; petioles 1 to 2.5 cm. long, glabrous, or the base slightly rusty puberulent. Flowers unknown. Fruit solitary, peduncles 1 cm. long, glabrous, the persistent calyx lobes six, in two series, the inner ones thinner than the outer, rounded, 5 mm. long, rusty pubescent, becoming glabrous on the outside, densely appressed rusty pubescent on the inside, the fruit one-seeded ovate or obovate, the apex rounded, and with a short minute mucro, smooth and shining, 2 cm. long, 1 cm. thick, the seed ovate acute, 1.5 cm. long, smooth and shining.

Type specimen No. 3744, Merrill, Mount Mariveles, Province of Bataan, Luzon, January 1, 1904.

A tree 6 to 8 m. in height, the trunk about 20 cm. in diameter, growing on exposed ridges at an elevation of about 1,000 m. above the sea. The bark has a small amount of milky sap which, however, is not utilized by the natives. A most distinct species especially characterized by its small and relatively very narrow leaves.

PALAEQUIM BATAANENSE Merrill, sp. nov.

A tree reaching a height of 45 m. with coriaceous, glabrous, obovate, acute or obtuse leaves, the nerves about nine pairs. Branches reddish brown, the tips more or less pubescent with rusty appressed hairs. Leaves pale when dry, 10 to 14 cm. long, 4 to 6 cm. wide, entirely glabrous, narrowed more or less from above the middle to the cuneate base, the apex obtuse or abruptly acute, rarely acuminate; nerves obscure above, not especially prominent beneath, at intervals of from 12 to 15 mm., the reticulations faint, lax; petioles glabrous, 1 to 1.5 cm. long. Flowers near the tips of the branches, solitary or in pairs from the axils of the leaves, or from the axils of fallen leaves, numerous, greenish white, the pedicels 1 cm. long, minutely and densely rusty pubescent. Calyx 4 mm. long, the three outer sepals broadly ovate, acute, densely rusty pubescent, the three inner ones thinner, pubescent only where not protected by the overlapping outer sepals, broadly obovate or rotund, the apex rounded. Corolla 14 mm. long, the six lobes reflexed in anthesis, 10 to 11 mm. long, 3 mm. wide, acute, glabrous. Filaments 4 to 5 mm. long; anthers 3 to 3.5 mm. long. Style exserted, 1.5 cm. long. Fruit unknown.

Type specimen No. 169, Forestry Bureau, collected by Barnes, Lamao River, Province of Bataan, Luzon, January, 1904; No. 156, Forestry Bureau, collected by Barnes, same locality, is apparently the same but with leaves much attenuated at the base.

A tree growing in the dry hill forests at an elevation of 100 m., the gutta-percha produced being of no value. Perhaps most closely related to *Palaequim luzoniensis* (F.Vill.) Vidal, but the leaves with few nerves, entirely glabrous, and the petioles comparatively short. In this species the corolla

is very persistent, while in all the others of the genus known from the Philippines, the corolla falls very readily.

PALAEQUIM TENUIPETIOLATUM Merrill, sp. nov.

A tree 18 to 35 m. high, with glabrous, lanceolate, acuminate leaves, the nerves about ten pairs. Branches slender, dark reddish gray, sometimes almost black, glabrous. Leaves lanceolate, or sometimes somewhat oblanceolate, 5 to 10 cm. long, 3 to 5 cm. wide, firm, entirely glabrous, or the midrib below, with few appressed, ferruginous hairs, usually rather abruptly acuminate, often caudate, the acumen slender, blunt, 1 cm. long or less, rarely nearly acute, the base cuneate; nerves not prominent, irregular, obsolete or nearly so near the base of the leaf; petioles slender about 1.5 cm. long, glabrous, or with few appressed, ferruginous hairs. Flowers fasciculate or sometimes solitary, on the branches below the leaves, the fascicles containing from two to four flowers; pedicels rusty pubescent, 3 to 8 mm. long. Calyx lobes 3 mm. long, 2 mm. wide, obtuse or somewhat acute, densely rusty-tomentose, the inner lobes thinner and somewhat exceeding the outer. Corolla greenish white, the tube about 2 mm. long, lobes lanceolate, 6 mm. long, 2 mm. wide, acute, reflexed in anthesis, glabrous. Stamens nine to twelve, the filaments 3 to 3.5 long; anthers 2 mm. long. Style exserted 1 cm. long. Ovary rusty-pubescent.

Type specimen No. 154, Forestry Bureau, collected by P. T. Barnes, Lamao River, Province of Bataan, Luzon, January, 1904; Nos. 516, 520, and 191, Forestry Bureau, collected by Barnes from the same locality, are the same, also No. 1991, Merrill, Pagbilao, Province of Tayabas, Luzon, March, 1903.

A tree 18 to 35 m. high, and 1 m. in diameter or less, with somewhat developed buttresses, extending to a height of 2 m. or less, and dark gray, nearly smooth bark which produces a thin milk sap, which flows rather freely, but which does not coagulate readily.

A species growing in dry hill forests, and apparently most closely related to *Palaquium lanceolatum* Blanco, differing from that species in its differently shaped leaves, longer petioles, absence of evident nerves near the base of the leaves, and dark-colored branchlets, the milk sap of the two species being also very different in quality. No. 1991 Merrill, has previously been referred to *Palaquium lanceolatum* Blanco.¹

EBENACEÆ.

DIOSPYROS COPELANDI Merrill, sp. nov.

A small tree 8 m. high and 8 to 10 cm. in diameter, with oblong, glabrous, coriaceous leaves, slender pendant branchlets, and entirely glabrous inflorescence which is borne in fascicles on the trunk of the tree. Pendant branchlets sometimes 2 m. long, 0.5 mm. in diameter, the bark gray, glabrous. Leaves 1 to 3.3 dm. long, 7 to 10 cm. wide, glossy above, dull beneath, the apex acute or abruptly, broadly, short-acuminate, the base rounded, subcordate, the nerves not prominent, freely anastomosing, the reticulations

¹ Merrill, Govt. Lab. 6:15. 1904.

lax, the main nerves about 15 pairs; petiole thickened, 0.5 mm. long. Staminate flowers 13 mm. long, glabrous, crowded in fascicles on the trunk of the tree, the peduncles 1, rarely two or three-flowered, glabrous, 6 to 8 mm. long, with a whorl of minute bracts at the base. Calyx 4 mm. long, 5 mm. in diameter, 5 to 6 lobed, the lobes broad, short, acute or rounded. Corolla flesh colored, 10 to 11 mm. long, 5 mm. in diameter, 6 or rarely 7 lobed, when open the lobes spreading or reflexed, lobes 3 mm. long, 4 mm. wide, rounded. Stamens about 25, inserted on the disc and the base of the corolla; filaments 1 to 1.5 mm. long; anthers lanceolate, glabrous 6 to 7 mm. long, dehiscing longitudinally, the rudimentary ovary glabrous. Female flowers and fruit unknown.

Type specimen No. 246, E. B. Copeland, Lamao River, Province of Bataan, Luzon, February, 1904. A species probably of the section *Ermellinus*, growing in hill forests along the river at an elevation of about 100 m. above the sea.

GESNERACEÆ.

TRICHOSPORUM CARDINALE Copeland, sp. nov.

Calyx 5-partite almost to the base, persistent. Seeds with one very short hair at each end. Corolla cardinal, curved.

No. 997, E. B. Copeland, in thickets along the trail to Mount Apo, district of Davao, Mindanao, April, 1904, altitude 5,000 feet.

A climbing vine, where the moisture-demanding epiphytes begin to dominate the forest. Stem glabrous. Leaves opposite, equal, subcoriaceous, glabrous, entire, acuminate, very pale beneath, the lateral veins obscure, 12 cm. long, 3 cm. wide, the petioles 6 mm. long. Flowers in clusters of two or three on almost obsolete peduncles, the hairy pedicels 1 cm. long, axillary and terminal. Calyx lobes linear, 5 mm. long, hairy. Corolla hairy without, 4 cm. long. Stamens didynamous, exerted, filaments glandular. Ovary linear, three times as long as the pubescent style. Capsule about 10 cm. long, curved. Seeds less than 1 mm. long, the single hair at each end shorter than the seed.

DICHTROTRICHUM GLABRUM Copeland, sp. nov.

Leaves broadly lanceolate, acute, obscurely serrate, glabrous to the naked eye, a very few hairs visible under the lens. Peduncles long. Calyx shallowly 5-toothed, the segments acute.

No. 998, E. B. Copeland, Mount Apo, district of Davao, Mindanao, altitude about 6,000 feet, April, 1904. No. 297, DeVore and Hoover, same locality, May, 1903.

A vine with pendent tips, in the mossy forest. Stem slender and almost glabrous. Larger leaves 10 cm. long, by above 3 cm. broad, on petioles 4 or 5 cm. long; veins about 6 on each side of the midrib. One leaf of each pair reaches a length of less than 2 cm. and soon falls. The pendent peduncles are 20 to 40 cm. long, and, like the less (in anthesis) than 1 cm. long pedicels, glabrous or nearly so. The showy cyme comprises about eight flowers, with curved, obscurely bilabiate red corollas about 2.5 cm. long. The campanulate calyx is about 5 mm. long, and divided not more than one-fifth of its length. The capsules reach a length of 25 cm. Seeds as in *Dichrotrichum ternateum* Reinw.

DICHBOTRICHUM CHORISEPALUM Clarke, in DC. Monog. Phanerog. 5:53.

Specimens of this species collected by W. Klemme, Mount Banajao, Province of Tayabas, Luzon (Forestry Bureau No. 888) differ from Clarke's description in having the leaves acute-serrate rather than dentate, not very sparsely hirsute, too abruptly contracted to be called cuneate, and with four instead of six prominent nerves on each side of the midrib. Peduncles 40 cm. long (E. B. C.).

RUBIACEÆ.

GARDENIA BARNESII Merrill, sp. nov.

A shrub or small tree with ovate-lanceolate petioled leaves, and large white fragrant flowers, 10 cm. long. Ultimate branches glabrous, slender, light gray, the terminal buds resinous. Leaves 8 to 15 cm. long, 4 to 7 cm. wide, both surfaces shining, the upper glabrous, the lower slightly pubescent on the nerves, apex short acuminate, sometimes acute, the base cuneate; nerves about 15 pairs, rather prominent beneath; petiole 1 to 1.5 cm. long; stipules caducous. Flowers solitary, axillary, the pedicel 0.5 cm. long, sometimes wanting. Calyx 2 to 3 cm. long, glabrous, the tube gradually widened upward, about 1.5 cm. long, somewhat cleft on one side, with two short broad teeth, four or five ridged, the ridges extending into as many linear obtuse ascending or spreading lobes 1 to 1.5 cm. long, 4 mm. wide. Corolla about 8 cm. long, the tube glabrous throughout, 5 to 6 cm. long, the limb 5 to 7 parted spreading, 6 to 7 cm. in diameter, the lobes obovate, rounded, glabrous, about 3 cm. long, 2 to 2.5 cm. wide. Stamens as many as the lobes of the corolla, the anthers 13 mm. long, partly exserted. Stigma 1 cm. long, three cleft. Fruit unknown.

Type specimen No. 163, Forestry Bureau, collected by P. T. Barnes at Lamao River, Province of Bataan, Luzon, January, 1904. A shrub or small tree growing in hill forests at an elevation of 100 m. above the sea, reaching a height of 9 m. and a diameter of 11 cm. The bark is light gray and the wood light yellow, hard.

A species of the section *Eugardenia*.



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No. 18.—OCTOBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES
BIOLOGICAL LABORATORY

PART I

AMEBAS: THEIR CULTIVATION AND
ETIOLOGIC SIGNIFICANCE

BY

W. E. MUSGRAVE, M. D., AND MOSES T. CLEGG

•

PART II

TREATMENT OF INTESTINAL AMEBIASIS
(AMEBIC DYSENTERY) IN THE TROPICS

BY

W. E. MUSGRAVE, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
1904

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LETTERS OF TRANSMITTAL

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., August 10, 1904.

SIR: I have the honor to transmit herewith a report on (1) Amebas: Their Cultivation and Etiologic Significance, by W. E. Musgrave, M. D., and Moses T. Clegg; and (2) Treatment of Intestinal Amebiasis (Amebic Dysentery) in the Tropics, by W. E. Musgrave, M. D.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY,
Manila, P. I., August 4, 1904.

SIR: I have the honor to submit herewith and to recommend for publication reports on (1) Amebas: Their Cultivation and Etiologic Significance, by Dr. W. E. Musgrave, Pathologist Biological Laboratory, and Mr. Moses T. Clegg, Assistant Bacteriologist; and (2) Treatment of Intestinal Amebiasis (Amebic Dysentery) in the Tropics, by Dr. W. E. Musgrave.

Very respectfully,

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PART I.

**AMEBAS: THEIR CULTIVATION AND ETIOLOGIC
SIGNIFICANCE.**

By Dr. W. E. MUSGRAVE and Mr. MOSES T. CLEGG.

SYNOPSIS.

I. Introduction.

II. Cultivation.

Review of literature.

Various media (including our own).

Cultural technique.

Bacterial symbiosis.

Pure cultures.

Cultivation of pure species of amebas.

Cultivation of pure species of amebas with pure cultures of bacteria.

Selectiveness of amebas for special bacteria which may be symbiotic with them.

Special symbiotic bacteria.

Distribution of amebas and sources from which they may be obtained by culture.

(a) Externally.

(b) Animals other than man.

(c) Man.

Cultural characteristics (general).

(a) Morphologic.

(b) Agglutination.

(c) Nutrition.

(d) Reaction to physical conditions.

(e) Reaction to chemical agents.

(f) Reactions to sera, blood, toxins, etc.

Cultivation of special amebas.

(a) Ameba 11524.

(b) Ameba 11147.

(c) Ameba "water."

(d) Ameba "lettuce."

(e) Ameba 25624.

(f) Ameba "G."

(g) Ameba 34354.

(h) Ameba 17786.

(i) Ameba 12485.

(j) Ameba 12483.

(k) Ameba "H."

(l) Ameba "L."

(m) Ameba "C."

(n) Ameba "urine."

III. Etiologic significance.

- (a) Amebas are harmless commensals.
- (b) They intensify or alter the lesions already present.
- (c) There are pathogenic and nonpathogenic amebas.
- (d) All amebas are or may become pathogenic.
- (e) Animal experiments.

IV. Concluding remarks.**V. References to literature.**

PART I.

AMEBAS: THEIR CULTIVATION AND ETIOLOGIC SIGNIFICANCE.

By W. E. MUSGRAVE, M. D., and MOSES T. CLEGG.

I. INTRODUCTION.

The cultivation of amebas,¹ while a subject of the greatest importance, has never excited a very general interest, especially in America; and, although there is an extended literature on the subject, it is scattered over a long period of time and unfortunately some of the earlier writings deal with work not performed with proper care.

This early and unsatisfactory work accounts partly for the small amount of attention which the more careful articles of recent times have received, and materially adds to the responsibility of those who now take up the subject. Some of the confusion and doubt which exist in regard to cultivation and etiology is also due to the fact that a number of those who have grown amebas have, although insufficient data were at hand, devoted much time to a discussion of the biology and classification of the parasites.

The controversy relative to the etiologic significance of these parasites in human disease has been active almost since the time of the very important observations of Lösch, and, to judge from much of the recent literature, is still far from being decided. Any attempts at the solution of these problems must take this doubt into consideration, and every detail of the subject must therefore be made clear.

¹The authors have followed Dorland's Dictionary in spelling.

In this first paper we have purposely avoided a detailed discussion of the life cycle, the classification, and other strictly biologic questions regarding amebas, but instead have taken up their cultivation from various sources, including the dysenteric intestine, and have proved their etiologic role by animal experiment with cultures of diverse origin, and have shown that at least some amebas are pathogenic. Certain of these pathogenic ones are found in places which indicate methods for the prevention of the infection, a consideration which is of great importance to us in the Philippine Islands.

The term "amebiasis," which we have introduced, is in line with modern nomenclature and is comparable in its application with uncinariasis, trypanosomiasis, filariasis, etc.; and indicates an infection with amebas. It is the only term thus far suggested which covers all grades of the infection and is readily adapted to the various locations in which such infection may be found. We need not here enter into a discussion of the defects of other names which have from time to time been advanced. It is almost universally admitted that none of them are entirely satisfactory and that many of them are inappropriate.

The general arrangement of the subject-matter has been indicated above, and it is given in this somewhat unusual manner because it meets the questions in the order in which they have appeared to us.

II. CULTIVATION.

Desultory attempts at the cultivation of amebas were probably made many years ago.

Aurbach (1856) asserts that he grew these organisms by exposing water containing a small piece of animal tendon to sunlight, but the more interesting and important observations are of a later date and follow Lösch's valuable work of 1875.

Cunningham (1879) cultivated amebas in boiled solutions of cow's dung and other substances. Grassi (1882) repeated and enlarged on Cunningham's experiments. Both of these authors believed that they had succeeded in growing *Amœba coli*.

Kartulis (1885) failed to grow *Amœba coli* in media composed of fluid blood serum or liquid gelatin. In 1890, with media composed of sterilized alkaline solutions of rabbit and pigeon dung, he believed he had been successful. His best media (1891), however, were either solutions of ordinary bouillon or sterilized alkaline decoctions of hay.

Kovacs (1892) repeated the methods of previous writers, but failed to cultivate dysenteric amebas by the use of any of them.

Ogata (1893) asserted that he had obtained pure cultures of protozoa by a unique method which depended for its results upon a principle now generally known as geotropism. He used as a medium a 2.5 per cent solution of grape sugar in sterilized tap water. After growth had been established he separated the protozoa by the use of a small glass tube, which was nearly filled with the medium and was placed in a vertical position with its lower end in the culture. By the action of the negative geotropism on the part of the protozoa they wandered upward into the small tube, and after various lengths of time Ogata obtained lengths, by sealing it off and removing sections, which contained only one variety of the protozoa, which could then be transferred to other media. He succeeded in transplanting some of his protozoa in gelatin media.

Vivaldi (1893-94) maintained that he had grown *Amæba coli* in sterilized straw decoction, and had produced diarrhea in cats by infection with these cultures.

Kruse and Pasquale (1894) repeated and criticized the previous work on the cultivation of amebas and concluded that *Amæba coli* had not theretofore been grown. By following the technique of others they obtained amebas, but neither by any of the previous methods nor by more elaborate ones were they able to obtain *Amæba coli*.

Miller (1894) cultivated, in the presence of other microorganisms, amebas in a variety of fluid media. One-fifth per cent milk in hydrant water, dilute bouillon, and hay infusions were considered the most satisfactory. Dilution of the media and a large exposed surface were thought requisite.

Celli and Fiocca (1894) in a preliminary report stated that they had grown amebas free from other microorganisms, with the exception of bacteria, on solid media. In a complete report which appeared in 1895 they described their methods in detail. They were successful in growing amebas in several media, including alkaline potatoes, ascitic fluid, and egg albumen, their most satisfactory one, however, being 5 per cent *Fucus crispus* in bouillon or water which was strongly alkaline.

Picardi, Perroncito, and Bosso (1894) claimed successful cultivation on agar media.

Fijardo (1896) cultivated in straw decoction media amebas from dysenteric stools, but did not consider them to be *Amæba coli*. Beyerinck in the same year grew amebas together with bacteria or torulae on several solid media. One species developed well on ordinary nutrient agar and gelatin.

Schardinger (1896-97) cultivated, on solid media, amebas which had been obtained from stools and other sources. The medium which he recommended was made from agar, hay, water, and calcium hydrate.

Peyrot and Roger (1896) cultivated amebas on media composed of straw infusions, but did not regard the organisms as *Amæba coli*.

Frosch (1897) grew amebas on solid media. He thought special media not to be so necessary to growth as satisfactory living bacteria. His *Amæba nitrophilia* grew well on ordinary agar provided the proper bacteria were present. He experimented with several media, among others, the *Fucus* of Celli and Fiocca, the agar of Beyerinck, the hay infusion of Schardinger, aromatic gelatin, potatoes, charred turnips, and beets. None

of these were satisfactory. He obtained good results with asparagin and glycogen solutions; but the simplest medium and the one most suitable for his amebas was composed of agar one-half per cent, bouillon 10 per cent, and tap water 90 per cent. On this the parasites always grew well when in the presence of bacteria.

Casagrandi and Barbagallo (1897) used both fluid and solid media, and claimed success with both. They also obtained positive results with the sterile white of egg, to which sodium bicarbonate had been added.

Jensen (1898) asserts that he successfully cultivated amebas from the intestine and other sources, on a solid medium, which was prepared from barley sprouts fifteen days old, which were cooked in water, filtered, rendered alkaline, mixed with agar, and autoclaved.

Taujitan (1898) grew amebas on solid media prepared from straw, *jigartina prolifera*, agar, and sodium carbonate. He also used with success the media recommended by Frosch.

Zaubitzer (1900) repeated the work of Celli and Fiocca as well as that of several other experimenters, and, although by these methods he succeeded in obtaining amebas from a number of substances, the results were not satisfactory. He recommended as more suitable media, solutions of somatose, either in liquid or as a 1 to 2 per cent somatose agar made alkaline.

Mouton (1902) was successful in the cultivation of amebas from garden earth. His medium was practically that used by Frosch and his results in general were confirmatory of that author's work. Mouton succeeded in securing his amebas in pure culture with one species of bacteria, and isolated a diastatic ferment therefrom.

Gottstein (1903) succeeded in cultivating amebas on solid media. He used Dietjen's blood platelet medium, on which a small variety of ameba grew very well; but another would not grow even when somatose, as recommended by Zaubitzer, was added. Besides this he used various combinations which had been previously recommended. The somatose-agar of Zaubitzer, in the proportions advised by that author, was not so successful in his hands; but when somatose was used in smaller amounts in combination with sodium chloride and agar the medium thus obtained proved very satisfactory. His most successful results, however, were secured with the dilute bouillon agar of Frosch.

Our own work on the cultivation of amebas has been going on for some years. We have from time to time repeated the work of the principal experimenters, but usually, when following their methods, with negative or unsatisfactory results, so far as the cultivation of amebas from the intestine is concerned.

Particular attention has been given to the *Fucus crispus* medium of Celli and Fiocca, but we have been unable to confirm their results. In following their directions regarding the preparation and alkalinity of the medium, we have been able to obtain only a semi-solid product, which was neither transparent nor colorless—prop-

erties so essential for plate work. We were able to grow straw amebas on it, but have never succeeded with those from the human intestine.

The somatose-agar advised by Zaubitzer has been somewhat more satisfactory. But, as stated by Gottstein and others who have repeated his work, we have found that to obtain favorable results, a much smaller proportion of the somatose than has been recommended should be used. Better results have been obtained with the bouillon-agar medium recommended by Froch and used by Mouton, Gottstein, and others. It has been especially satisfactory when symbiotic bacteria have been present.

It would be useless to describe all the various media which we have employed. Amebas from water, hay, soil, etc., may be grown indefinitely on a large variety of them, but for the cultivation of those which have passed through the alimentary canal of man and other animals the choice is not so great.

As a stock medium we have come to use the following preparation:

Agar	20.000
Sodium chloride300 to .500
Extract of beef300 to .500

Prepare in the same manner as ordinary nutrient agar. The finished product is most universally satisfactory when 1 per cent alkaline to phenolphthalein.¹

Variations from this stock medium, consisting in the use of a still smaller amount of sodium chloride and beef extract, or in leaving out the salt entirely, will sometimes be found advantageous, especially when the amebas are growing in company with a very luxuriant saprophytic bacterium. On the other hand, a very small

¹ In order to obtain a final reaction of 1 per cent alkaline to phenolphthalein it will usually be found necessary to start with an initial alkalinity of 1.5 per cent. After autoclaving the medium in a flask, filtering the precipitate, distributing in tubes, and autoclaving again, the final product will be clear and will have an alkalinity of about 1 per cent.

Wherry (Bulletin No. 19 of this Bureau) has recently discussed the precipitation of acid albumins in alkaline media and its effect upon both the reaction and the nourishing properties of the finished product before and after the removal of these substances. We have found that our media are more satisfactory when these precipitated albumins are filtered off, although by this method the nutritive value for bacteria is somewhat diminished.

quantity of peptone sometimes improves it, when a very delicately growing bacteria is being used. When this is desirable the simplest way to prepare the medium is to use from 5 to 10 per cent of ordinary laboratory bouillon and 2 per cent agar in water.

Technique (diagnostic).—Before continuing the discussion of the results we obtained by cultivating the protozoa a description of methods for the study of amebas, separated either from the feces or from cultures, should include a consideration of fresh preparations and of the various staining reactions. The course to be followed in determining the presence of amebas in the feces has been so universally described in text-books and the literature in general that repetition here is not essential.

There are, however, two points in this connection to which we wish to call attention. The first is the error of the general recommendation to select from a stool a mass of mucus as most advantageous for microscopic examination, because it is supposed to contain a larger number of organisms than material from other sources. *To get the best results the patient should always be given a saline cathartic and the examination should be made from the fluid portion of the stool.* Salts not only make the examination easier and the results more reliable, because of the fluid stools which they produce, but in addition they give rise to a larger number of parasites in the specimen, owing to the fact that in such a procedure the entire colon is washed and amebas which may be propagating in it in various locations are thus flushed out; whereas in the examination of a natural passage, even though the patient has diarrhea parasites from the cecum may not be passed.

In the second place, the diagnosis of amebas should never be made unless they are in a motile state. Errors are often committed in this way, even by the experienced. Ordinarily one may be reasonably certain of the diagnosis of typical resting or encysted forms; yet even here mistakes are occasionally made. In the interest of the patient, further examinations should be insisted on after the use of a saline cathartic.

In addition to the examination of fresh specimens, many other methods of diagnosis have from time to time been suggested. Probably none of them have any especial advantage, if indeed they are as certain as the use of the fresh material. A few of them will be noted.

Zorn recommends the following method for preparing stained and permanent preparations:

A few cubic centimeters of feces are mixed with three or four volumes of a solution consisting of fifteen parts of 1 per cent solution of chromic acid and three parts of 1 per cent solution of osmic acid. The mixture is shaken thoroughly and after ten minutes is centrifuged. The sediment is then mixed with five volumes of a 25 per cent solution of Beale's carmine, allowed to stand for one-half hour and again centrifuged. The sediment is then washed in a weak, rose-colored solution of the same carmine and mounted in glycerin, or first dehydrated and then mounted in balsam.

Doflein recommends for fixing either of two solutions:

I.—

Saturated aqueous solution $HgCl_2$	100
Alcohol	50
Acetic acid	5

II.—

Picric acid	2
Alcohol	50
Acetic acid	5

According to this author, the protozoa may be fixed as a thin film spread on a slide, or they may be handled in bulk, embedded in paraffin and treated as sections.

For staining, the following dyes may be used: Borax carmin, Grenacher's hematoxylin and eosin, gentian violet, saffranin, Heidenhain's iron hematoxylin, or Romanowsky's methylene blue and eosin.

A number of writers have spoken of staining *in vivo* by neutral red and a few other substances.

By running a dilute solution of neutral red under a cover-glass preparation from a culture, beautiful pictures may be obtained. (See figs. 24 and 25.) When young motile amebas are present they take up the stain without interfering with their motility for considerable periods of time, and while there is not a great deal of differentiation, the bright color makes it possible to follow the parasites closely and to observe the character of their movements. This stain will also give a fair picture of resting amebas, but for encysted ones it is useless.

For staining permanent preparations of amebas from culture the most satisfactory method we have yet used is Wright's modification of that of Romanowsky, and the technique is the same as the

one recommended for blood films. By applying this method to fresh cover-glass impressions from plate cultures, the amebas are often fixed in various phases of motion and the general structure is very well shown. It does not as a rule give a satisfactory nuclear picture. (See figs. 7, 8, 9, and 10.)

Craig has recently, by a modification of this method (not yet published), succeeded in staining amebas from the stools in such a manner as to bring out all the details. He maintains that he has been able to demonstrate by this means that *Amæba coli* multiplies by sporulation, a belief which also has been held by several other writers since Cunningham, some of whom, including Schaudin, have claimed its demonstration.

The known methods of staining have not been so satisfactory in our hands as could be desired, and any improvements in this particular line will, no doubt, yield valuable results when applied to amebas in culture.

Technique (cultural).—The media and some of the methods to be employed have already been discussed; but attention must also be given the methods of securing bacterial symbiosis and of isolation as well as to other points. Throughout these experiments the technique used to prevent contamination, etc., has been the same as that employed in bacteriologic work.

In obtaining cultures from water and from most other external sources, a large sample of 100 to 500 cubic centimeters of water, or of an aqueous solution or suspension of other substances, is collected in a cotton-plugged, sterile flask, to which is added 0.5 to 1 cubic centimeter of ordinary 1 per cent alkaline bouillon to each 100 cubic centimeters of sample. The flask is then set aside for from twenty-four to seventy-two hours, when an examination will usually reveal amebas on the surface of the liquid. A loop of this culture is then spread over the surface of a Petri dish upon which the melted medium recommended in another place has been poured and allowed to harden. In the course of from six to forty-eight hours the plate should be examined under a low power of the microscope, as is done in the study of bacterial colonies; the amebas can then readily be seen and followed. Finally, the culture may be transferred from plate to plate or inoculated on ordinary slants of the same medium.

As a rule, with cultures from such external sources little or no care will be necessary in regard to satisfactory symbiotic organ-

isms, as amebas are less selective under such circumstances and suitable bacteria are carried over in sufficient numbers to nourish the parasites. In isolating them from stools, however, much more care is necessary in this respect. We have never been able to obtain amebas from this source by first inoculation into fluid media of any kind. In plates prepared according to the preceding recommendations we have occasionally succeeded in securing growths simply by lightly smearing the surface with material selected from feces containing amebas. Success in such cases probably means that satisfactory organisms have been carried over in sufficient numbers and have been so distributed as to give the parasites nourishment. Positive results by this means, however, are not constant, but fortunately there is opportunity for improvement.

Owing to the selectiveness of amebas for certain bacteria, and particularly of amebas from the intestine on their first transplant, in order to grow them with more constancy, the surface of several plates should be smeared with pure cultures of various kinds of bacteria and then inoculated with the intestinal contents. It will be found that amebas will develop upon one or more of these plates while showing no evidence of doing so on any of the others. There are no means of determining beforehand what organisms will be satisfactory to an artificially uncultivated ameba, but by using a variety, comprising from six to twelve species, the percentage of positive results can be very much increased. By the use of twelve different selected bacteria we have succeeded in obtaining growth in 30 per cent of one series of cases, where the control inoculation made on the same medium without bacteria showed only 2 per cent of positive results.

Where it is desired to cultivate amebas from any particular intestine, and the means already mentioned have been tried but have failed, there is still a possibility of obtaining growth by first making cultures on ordinary agar plate from the same intestine and using the bacteria which develop as symbiotic ones when the material containing the amebas is added. By this method we have grown amebas in three out of five cases, where all other methods had failed.

Amebas containing red blood cells probably do not reproduce, and it is possible that there are other conditions of environment or phases of the life cycle which play an inhibiting part in certain cases. In one instance we have succeeded in obtaining a sat-

isfactory growth by first causing the amebas to encyst through exposure for twelve hours in the ice box, when all of our cultures from the specimen, which had not been treated in this way, failed to develop. In other instances we have grown the protozoa from stools in which they could not be positively demonstrated by careful microscopic examination.

The first plates taken from the stool or from the intestinal ulcers must be watched frequently and carefully under the microscope, and as soon as it is found that amebas have developed (which they do after a period varying from twenty-four hours to four or even five days) transplants must be made. If this is not done promptly the parasites are liable to die, and no further growth will take place. When the amebas have once become accustomed to the artificial media, however, less trouble will usually be found.¹

Symbiosis with bacteria.—Almost since the time when the first attempts at the cultivation of amebas were made, bacteria have been found associated with them, and much time has been devoted to the study of methods looking to the separation of the two classes of organisms.

Baumgarten (1890) and others before and since that time have pointed out the probability of coöperative action between amebas and bacteria in the production of dysentery; and this was followed by suggestions that the association of the animal parasites and bacteria was not an accidental condition, but a true symbiosis. More extensive and satisfactory experiments in cultivation, based upon the supposition that this symbiosis is necessary to the life of the protozoa, have been developed in recent years.

Janowski (1897), in one of the best reviews of this subject which we have seen, says, that "it is not improbable that the symbiotic organisms may determine the pathogenicity of the amebas themselves, and that the two may be associated outside and carried together into the alimentary canal, or that the former (bacteria) may already be in the intestine."

Views less far-reaching but similar in trend have been held by many of the most careful workers both before and since Janowski's observations were made. Prominent among these Beyerinck, Schardinger, Frosch, Mouton, Zaubitzer, Gottstein, and several others may be mentioned.

The existing data upon the extent and importance of this question will naturally be brought out in the discussion of attempts to obtain pure cultures of amebas.

Pure cultures.—Many methods have been employed for this purpose, but few if any have ever been successful; and even in those

¹In keeping stock cultures, transplants have been found successful with some amebas after six and one-half months, while with others they have been found to die unless much more frequently transferred.

cases where the parasite was supposed to have been isolated, its satisfactory development never took place when transplants were made.

Kartulis (1890) thought that, on a medium consisting of a straw decoction, he had obtained a pure culture of amebas from an abscess of the liver culturally free from bacteria.

Ogata (1893) maintained that he had secured protozoa in pure culture. His method consisted essentially in taking advantage of the principle now quite generally known as negative geotropism, which is a movement peculiar to a number of the protozoa.

Celli and Fiocca (1895) attempted to eliminate the bacteria by various methods, such as exposure of the mixed cultures to 55° and 60° C. for various lengths of time, filtration, placing disinfectants in the media, and inoculation into animals, thus endeavoring to secure pure cultures. But when at length, after great pains, they succeeded in obtaining amebas free from bacteria, the former did not reproduce on transplants.

Casagrandi and Barbagallo (1897) concluded that it was impossible to separate the amebas from the bacteria in the ordinary media. By the use of the *Fucus crispus* medium of Celli and Fiocca they secured cultures free from bacteria. They also succeeded in isolating amebas from a culture made with yeast. These writers revert to the original idea that the association of the protozoa and bacteria is an accidental one and that the life and propagation of the former are not dependent upon the presence of the latter.

Frosch (1897) took old encysted cultures of his *Amæba nitrophilia* and treated them at room temperature for from seventy-two to seventy-four hours with a 20 per cent solution of sodium hydroxide. This process he proved by cultural experiments destroyed nonspore-bearing bacteria but not the ameba cysts.

The latter grew when transplanted to media inoculated with suitable bacteria, but without, they always failed to develop. It then occurred to Frosch that certain products of the bacterial life might fill the requirements and that in this manner it might be possible to obtain pure cultures. With this object in view he used various filtrates from growing cultures and from digested bacteria, but all of his experiments proved negative. He therefore concluded that his *Amæba nitrophilia* was not a saprophyte, but was dependent for life upon nourishment found only in living micro-organisms.

Tsujitani (1898) obtained amebas in pure culture together with cholera vibrios by methods which will be mentioned presently, and then destroyed the latter by heating at 60° C. for forty minutes. He thereupon planted the encysted amebas in sterile media, where, although they became active, they did not multiply; but multiplication did take place when they were transferred to pure cultures, or to killed cultures of certain bacteria. We may therefore say that he had a pure culture of amebas. He arrived at the same result by another method. Sterile silk thread was dipped into a culture containing amebas and cholera bacilli and afterwards dried in the dessicator. By this process the cholera bacilli were killed, but the

amebas remained as cysts and were again placed on media with bacteria, where they developed in pure culture. Transplants on sterile media, however, were not satisfactory. Tsujitani concluded that when amebic cysts free from other organisms are inoculated on sterile media, they develop to the vegetative stage but do not further multiply, but that when living bacteria are added a luxuriant growth occurs. Living bacteria, therefore, form the most suitable food for these protozoa, but dead bacteria may also nourish them, as proved by the following experiment. Tsujitani took old cultures of a favorable symbiotic organism, heated them for one hour at 60° C. and then plated them to see that all the organisms were dead. These dead cultures were then inoculated with ameba cysts and development occurred, though not so luxuriantly as with living bacteria.

Zaubitzer (1901) by means of a 20 per cent solution of sodium hydroxide as recommended by Frosch, killed the bacteria occurring in encysted cultures of amebas on straw infusions. These cysts grew when transplanted to media inoculated with a suitable living bacterium, without the presence of which it was impossible to obtain a growth upon the same or any other medium. Killed cultures of bacteria were also employed, but without success. He therefore concluded that living bacteria are essential to the life of the parasites.

Mouton (1902) failed to secure amebas in pure culture and concluded that bacteria were necessary to the propagation and nourishment of the parasites.

Several others have attempted to grow amebas in pure culture by mechanical and other means, but the methods above described are the most important. With the possible exception of Tsujitani's work, which has not been confirmed, no one as yet has succeeded in obtaining satisfactory pure cultures of amebas which continue to develop on successive transplants.

Following the methods of Frosch, after making controls, three-month-old encysted cultures of three of our amebas ("11147," "11524," and "water") were washed with a 20 per cent solution of sodium hydroxide and then allowed to stand for seventy-two hours. Transplants were then made on sterile media and on media inoculated with organisms favorable to the growth of the amebas. Cysts were readily demonstrated in the alkaline solution at the time of transplanting as well as on the inoculated cultures at the end of twenty-four hours; but no growth had occurred. It was thought possible that in making transplants a sufficient amount of the strong alkali might have been carried over to prevent growth; and in order to obviate this contingency a second set of cultures was made after carefully neutralizing the alkali with hydrochloric acid, but without altering the results. We also varied the time of exposure to the alkali, and the strength of the solution; but we have never succeeded by this method in obtaining any development of amebas on transplants after bacteria have been destroyed. The

principal work done by others along this line has also been repeated by us.

Certain old cysts of amebas have been found to be more resistant to heat than are some of the bacteria, and after much work we have succeeded in destroying the latter without killing the amebas, as evidenced by the fact that the former grew on being transplanted to media which were simultaneously inoculated with another satisfactory symbiotic organism. The absence of the first organism from these transplants was determined by plate culture. Transplants of any of our amebas to bacteria-free media did not induce growth.¹

We have employed many other methods, including those of other workers, as well as a considerable variety which have not been previously described, but always with negative or doubtful results.

The only environment in which amebas are found to be apparently free from bacteria is that furnished by certain culturally bacteria-free liver abscesses. If these abscesses in reality do not contain other organisms, it probably proves that there are substances which are capable of nourishing amebas under certain conditions and which are not microorganisms. What is already known would naturally lead us to look upon such substances as ferments or enzymes.²

As we shall see below, amebas combined with various symbiotic bacteria in pure culture, if injected directly into the liver, cause abscess, in which both organisms may be found on exploration. We have recently made attempts to utilize this fact in a practical way. For this purpose, for instance, a monkey was first immunized against cholera spirilla, and after immunity had reached a high degree, a pure mixed culture of amebas and *Spr. cholerae* of the same stem as that used to give immunity was injected directly into the liver. The animal was killed on the fifth day after injection, but no abscess was found at necropsy, though the inoculation wound in the liver could be plainly seen. Smears from the liver did not show either amebas or bacteria. The control monkey, which received the same treatment without immunization, had an abscess at necropsy which contained amebas and *Spr. cholerae*.

¹The method mentioned above of drying cultures on silk threads has not been repeated by us up to the present time.

²Richet has shown that the normal liver contains a proteolytic enzyme in not insignificant quantities; and with this fact in view we now are experimenting with hopes of securing cultures free from living bacteria.

Various other attempts have been made to reproduce artificially a condition similar to that present in bacteria-free liver abscesses with the hope of securing satisfactory pure cultures; but they have invariably been unsuccessful.¹

It will be seen therefore that satisfactory pure cultures of amebas have not yet been obtained. The work of nearly all recent authors, as well as our own, *seems* to point to the impossibility of such a procedure, because a satisfactory symbiotic living microorganism is indispensable for the nourishment of these protozoa. However, failures to accomplish this purpose, so serious from a practical standpoint, do not prevent us, as we shall show in this paper and subsequent ones, from proving the etiologic significance of these parasites, by taking advantage of well-regulated symbiosis with bacteria.

The cultivation of a single species of ameba.—Having demonstrated that amebas from various sources will grow on a variety of media in the presence of other microorganisms, and having indicated the most satisfactory medium thus far known for this purpose, and finally having shown that amebas fail to propagate by any known methods in the absence of other microorganisms, or at least of organic life in some form, and that there are good reasons for believing such organisms or life really necessary for the growth of these protozoa, we are in a position to take up the next logical step in our work, namely, the study of methods for obtaining in culture a single species of ameba. For this purpose, in order to be accurate it will be necessary to start transplants from a single individual. Various methods, mostly mechanical, have been employed to this end.

Celli and Fiocca (1895), so far as we have been able to ascertain, are the first who called attention to this necessity; they obtained their results

¹We have destroyed the bacteria in pure mixed cultures of amebas and *Spr. cholerae*, by producing Pfeiffer's phenomenon in the abdominal cavity of a guinea pig. Amebas present in the fluid withdrawn after this operation are always encysted or destroyed, and no growth has ever been obtained on sterile media when the reaction was complete. On one occasion, however, we have been able to obtain a growth on media which were inoculated with another satisfactory symbiotic bacterium; and future experiments may determine this to be a useful method within certain limits for transferring the parasites from one bacterium to another. When old encysted cultures are used, it is probably the best way so far devised for obtaining amebas free from bacteria.

mostly by the use of a mechanical finger. Others following them have used methods largely dependent for their success upon the same general principle.

Beyerinck and others maintain that they were successful in accomplishing the same purpose by varying the character of the media. This method, however, while it might allow of a separation of two species, does not prove either of them to be pure.

Ogata's method of obtaining pure cultures in capillary tubes might be of advantage, the requisite being to find one of the small sections which contains but a single ameba.

However, when plate cultures are properly made, so that a very scattering growth is obtained with isolated amebas (as may often be seen) at a considerable distance from one another, the mechanical process is not a difficult one.

The following procedure has given us greater satisfaction than any other: Select a plate culture on which the parasites are well distributed, and after removing the cover, place the plate with the open side up on the stage of the microscope. By searching the edges of the growth with a Zeiss AA objective, places will be found where the amebas are some distance apart (1 to 3 fields). After locating a satisfactory parasite, which should be one on the surface of the medium, as practically all of them are, and having determined that there are no others in the field, either on the surface or at a depth, swing a clean and perfectly dry DD lens in place and gently lower it until the entire surface is in contact with the medium. Raise the lens quickly, swing in the AA objective, and determine whether the ameba is still present or has been picked up by the DD objective. If it has been picked up, which after some practice may be done two or three times out of five, the lens to which the ameba adheres is removed and, by gently rubbing its surface over that of a plate containing the hardened medium, the organism may be transferred. In this manner a pure culture, so far as amebas are concerned, may be obtained from the multiplication of a single parasite. The possibility of picking up more than a single individual by this method may be offered as a criticism, but with attention to the details which have been given, as well as to those which will suggest themselves to the worker, this method is certainly less open to criticism than the one involving the use of mechanical fingers or any of the other ones previously described. That only one ameba has been carried over by this method may still further be verified by examining with an AA objective the closed inverted plate on which it has been inoculated.

Another useful result of a careful application of this method is the aid it gives in obtaining pure cultures of amebas and of a single bacterium. The lens, of course, picks up the bacteria from a small field immediately surrounding the amebas; and as such isolated amebas are often surrounded by one kind of bacterium only, with the aid of a careful bacteriologic technique the pure cultures desired may sometimes be obtained in this manner. Methods of cleaning the lens and of obviating the entrance of air organisms readily suggest themselves. We have used the preceding method in most cases and have found it satisfactory. It would be possible to obtain the desired results more easily and with greater constancy by means of Unna's bacterial harpoon or a specially constructed lens, with a short adjustable focus and a cup-shaped extremity, like the marking arrangement which has been suggested for locating special fields in permanent preparations.

Cultivation of a single species of ameba with pure cultures of bacteria.—Having secured cultures of amebas of a single species, we are prepared to discuss the bacteria and methods of securing satisfactory symbiosis. A number of authors have reported the isolation of amebas with a single variety of bacteria; but apparently, very few have extended their work to include the isolation of a single species of the protozoa with a pure culture of a single kind of bacteria, the "pure mixed culture" of Frosch.

Beyerinck (1896), by allowing certain microorganisms to develop on plates, then inoculating the mixed cultures of amebas and bacteria at one point and watching the rapid development of the amebas, was able at times, to secure transplants which contained the desired bacteria and amebas only. By the same method he also isolated them with pure cultures of yeast.

The spread of amebas on the surface of media already inoculated with satisfactory organisms has been utilized by several others for the purpose of isolating the former with a single species of bacterium. With a slight difference in the mode of execution, this was one of the means employed by Tsujitani, Gottstein, Schardinger, Mouton, and others.

Some of the above-described methods for obtaining pure cultures, while failing in the specific object sought, were successful in isolating amebas in conjunction with a single organism. These procedures include the destruction of the bacteria in encysted cultures by heat, chemicals, and other substances, without eliminating the ameba cysts, which grow when transplanted to satisfactory media which have been simultaneously or previously inoculated with a suitable microorganism in pure culture.

In accordance with this principle, Zaubitzer, having satisfied himself that in contradistinction to bacteria, encysted amebas are not destroyed by 20 per cent sodium hydroxide, used this fact as a means of obtaining amebas

in culture with a single organism. After treating the encysted cultures with the soda solution, transplants were made to satisfactory media, which were also inoculated with pure cultures of the organisms desired. He obtained a satisfactory growth of amebas with *Spr. cholerae*, *B. typhosus*, *B. coli*, and other organisms.

Mouton (1902) secured amebas with one variety of bacteria by inoculating mixed cultures in plates which had been previously prepared by introducing the desired bacteria in lines radiating from the center. The amebas followed these lines more rapidly than did their associated bacteria, and were sometimes found with only the desired organisms. He also accomplished the same results by distributing colonies of the desired organism on plates of media, inoculating the latter with amebas which, as they wandered from one to the other of the bacterial colonies, lost the original organisms and eventually could be found pure with the desired bacteria.

Gottstein (1903), after inoculating the surface of plates with pure cultures of bacteria, brought upon the center a culture of amebas. These organisms then spread outwards until they reached the margins, and from this location it was possible to secure a pure culture with a single bacterium.

A satisfactory method of cultivating a single species of amebas with pure cultures of bacteria is, of course, absolutely necessary in order to accomplish reliable results. In repeating the work of others, we have found most of their methods tedious and uncertain. After many attempts the following satisfactory and quite simple routine has been developed, and it is now in daily use in our work. It is based on the principles maintained by Beyerinck, Mouton, and others, although simpler and more certain in its results.

The sterile ameba medium is melted and poured into ordinary Petri dishes, the usual precautions being taken. The dishes are then allowed to cool and the medium to become thoroughly hardened. With a platinum loop several rings of pure culture of the organism with which it is desired to grow the ameba are made on the surface of the hardened agar, and a small smear inoculation of the mixed culture of the amebas is placed in the middle of the smaller or central bacterial ring. (See figs. 1 and 2.)

If the necessary precautions have been taken, most amebas, as they multiply, will quite generally spread rapidly over the plate, and in passing through the rings of growing bacteria they will lose the organisms with which they started and take up those forming the rings. In from twenty-four to seventy-two hours the protozoa will have passed one or more of the rings, and from such locations they may be taken for transplanting. It sometimes happens that they appear on the first plate in pure cultures with the desired

organism, but more generally one or more transplants to the same medium are necessary before this end is reached. The further inoculations are made with amebas obtained from outside the largest ring on the next preceding culture.

This method is simple in execution, and the whole process may be watched under the microscope by inverting the plate and using a low power, following the process used in studying colonies of bacteria. With a Zeiss objective AA and ocular 4 the wanderings of the amebas and even their multiplication can be kept under observation.

The ring-shaped smear of bacteria has several advantages over one covering the entire surface of the plate. In the first place, amebas develop more rapidly by its use, and, secondly, they lose the original organisms much more readily than when moving constantly over a bacterial substratum.

Another feature which commends this method is the readiness with which it lends itself to a determination of the symbiotic value of a given organism. If for any reason it is not satisfactory to the amebas, they will not mix with or cross the bacterial rings. In some instances, where the organism is particularly unfavorable, the amebas, after wandering up to the inner margin of the first ring, encyst, and no further progress is made. On the other hand, where the antipathy is less marked, the progress is simply delayed until the bacteria carried over in inoculating the amebas have mixed with or crossed the ring, whereupon the amebas follow them.

When amebas have been isolated and grown in pure culture with a satisfactory symbiotic organism, it is sometimes difficult to transfer them to another. This is best overcome by first cultivating the protozoa for a short time on mixed cultures of the two organisms and then isolating them with the desired one by the use of the method described. Even by this means success is often doubtful and sometimes impossible of attainment, probably because the organisms with which the amebas are already associated are much more satisfactory than are those with which it is desired to have them continue.

Another difficulty is found in transferring the amebas from a very profusely and rapidly growing organism to one which is more delicate. Fortunately, but few grow profusely on the medium recommended, but where such a difficulty arises, a medium in which the amount of nutriment is still further restricted will prove

useful. This hindrance can further be overcome by allowing the rings to grow for from twenty-four to forty-eight hours after inoculation before placing the amebic culture in the center.¹

The number of bacteria to be carried over by the inoculation of the amebas can always be greatly limited by selecting on the margin of the growth of a previous plate culture the place from which to make the transplant.

Selectiveness of amebas for special bacteria which are symbiotic with them.—Amebas show a selective action for certain bacteria; this is true whether the culture is pure or mixed. This fact has been noticed by several of the more recent writers on cultivating these protozoa, including Beyerinck, Frosch, Tsujitani, Zaubitzer, Mouton, Gottstein, and others, all of whom have advanced evidence to this effect. When we add to these observations the facts which have been brought out by our own work, we are justified in concluding, at least tentatively, not only that living bacteria are necessary to the life of amebas, both in the animal economy and under other conditions, but also that there is a natural selectiveness on the part of the parasites for certain species of bacteria.

This selectiveness is particularly marked in amebas from the human intestine and other parts of the animal economy, and is equally true whether they have been brought to these places by natural means or by experiment. This is shown by the difficulty with which amebas from such sources can be cultivated, success seeming to depend largely on placing the proper organism with them in sufficient numbers. When taken from these surroundings they often develop only on plates which have been treated with a particular culture. This holds true even when the bacteria which are used are cultivated from the intestine of the case which harbored the amebas. However, after the latter have been grown for some time on artificial media, they become less selective and may finally be made to develop in symbiosis with any one of a large variety of microorganisms.

¹There is one difficulty sometimes encountered which it is impossible to overcome. Sometimes there are obtained in cultures certain very small amebas which do not move rapidly enough on the media and so reach the resting stage too early to allow of their separation from the bacteria. We have one such culture in our collection. It grows well on transplants, but we have so far entirely failed to secure it in pure culture with any organism.

Amebas, whether found in the intestine or outside the body, are practically always associated with various kinds of bacteria; but it does not necessarily follow that all of these play a part in the metabolism of the parasites. This is indicated by the microscopic examination of amebas taken from stools and other places, when they often show bacteria embodied in their protoplasm; but usually there is no indication that more than one kind is present in any young, healthy, growing individual, although a great variety may exist in its environment.

These conclusions are still further corroborated by methods of culture. If plate cultures are made from a substance in which amebas are multiplying, and all the various bacteria are isolated in pure culture and transplanted to our media, it will be found that the amebas will multiply profusely in some, will grow very indifferently in others, while in certain ones no growth whatever will occur. If amebas from other sources are inoculated into control cultures of these organisms, similar results will be obtained, excepting that the organism producing a satisfactory symbiosis and therefore a satisfactory culture of the second amebas, may be one entirely different, morphologically, culturally, and even in pathogenic action, from the ones suited to the amebas from the first source. *In a word, amebas in a natural environment are selective; but this property may be overcome to a certain extent on artificial media, and perhaps also in ways which are more natural. On the other hand, this phenomenon is reversible, for, by passing the amebas through the animal economy, the selectiveness may be increased.*

One of the most important applications of these results, when more is known of the cultural characteristics of the amebas, may lie in the fact that a knowledge of this will enable us to classify amebas and also to obtain them in pure species. Amebas which are alike, but taken from different natural environment, may be found to grow in symbiosis with identical organisms and may reject others which are also identical, while, on the other hand, other varieties may choose different bacteria for symbiosis and satisfactory growth, and, thus, a biologic difference may be established. However, unfortunately, the ease with which amebas may be made to change their habits, and, hence, the almost utter impossibility of showing their previous pleasure in this respect, throws considerable doubt upon the hope of any success in the direction suggested.

If, as has been shown, it is only with great difficulty that an ameba from an animal source can be grown, although it may be made to decrease its selectiveness by successive transplants on artificial media and may subsequently develop with any one of a variety of organisms, it becomes important to determine whether this process may be reversed. Repeated observations have, as stated above, proved that it may be, and that in so doing another element, which is not so easy of explanation, is encountered, namely, an acquired resistance to culture, apparently due to causes other than the lack of a satisfactory bacterial environment or symbiotic relationship.

It is easy to separate an ameba from Manila tap water which at first will grow with a large variety of organisms. If it is isolated with one variety and encysted cultures are obtained and injected, either subcutaneously or into the liver of an animal, abscess usually results, and the amebas from this abscess may again be grown with cultures of the introduced symbiotic organism and sometimes with other organisms. However, if the contents of this abscess or even cultures from it are transferred to another animal, it will be found more difficult to obtain growth from this second one; and this difficulty will increase as successive animals are used, until it becomes as great or greater than that attendant upon growing amebas obtained from the dysenteric intestine of man. We have repeatedly failed to obtain a growth in the second and third animals, although the amebas were microscopically present in the abscess contents and we were sure that the original symbiotic organism was being used.

These facts make us reasonably certain that another element has entered into consideration in the nutrition of amebas, and one which becomes most markedly manifest when the liver is used as the seat of the abscess. In a measure we have here conditions analogous to those present in liver abscesses in the human body, for from these also we have not succeeded in cultivating amebas in three cases with which we have had the opportunity of experimenting. A quite plausible explanation of this phenomenon is tentatively advanced by us, namely, that by these successive inoculations we have increased the selectiveness of the parasite to a point where the bacterial symbiosis is replaced by something else. In searching for the probable nature of the substance selected, enzymes or ferments

suggested themselves, some of which are known to be peculiar to the liver.

These facts render it possible that living bacteria are not really necessary to the life of amebas under all conditions. The trend of our work at the present time is corroborative of the foregoing results, and this further investigation will be embodied in another report, together with a discussion of the biology of the parasites.

There is still another question of importance in regard to this selectiveness and its variability, the answering of which is of the utmost importance from several points of view, namely, does the varying symbiosis, in addition to increasing the difficulties of cultivation, influence in any way the pathogenic nature of the parasite? For example, is an ameba in symbiosis with *Spr. cholerae* more or less pathogenic than one after a long symbiosis with a harmless saprophyte? At the present time we are only able to state that we have produced experimental dysentery in monkeys with the same kind of amebas which at one time were in symbiosis with *Spr. cholerae*, and at another with an absolutely nonpathogenic saprophyte, with no appreciable differences in the results; and furthermore, neither at the time when the dysenteric symptoms developed nor at necropsy were we able to obtain by culture the bacteria introduced with the protozoa. This would tend to support the view originally suggested by Janowski, namely, that it is not the nature of the first symbiosis but that of the one formed with the bacteria already in the intestine, which determines the pathogenicity, if indeed, the nature of the symbiosis is at all a factor in this question.

Whatever influences such habits may have on the pathogenesis, all the work which has been done points to the fact that it is the symbiosis formed *after* the introduction of the parasite into the intestinal canal which insures the propagation of the amebas there; and there is a great temptation to explain some of the negative results which have been obtained in experiments by the supposition that in the intestine of man or animals symbiotic bacteria satisfactory to the entering ameba were not present at the time of its introduction.

Having seen that such intestinal symbioses are formed in experimental animals, after feeding "pure mixed cultures" of amebas, and that it is just as difficult to reclaim the amebas from such intestines as it is from the human dysenteric ones, and finally that

the organism most likely or satisfactory for such cultural symbiosis is not necessarily the one introduced, but one of many which may be obtained from the intestine itself by culture, we are brought back to a proposition already mentioned, namely, that it may not always be the bacteria themselves which are necessary for culture or pathogenicity, but it may possibly be some ferment or bacterial product which produces this change.

The following table indicates the selectiveness of three of our amebas after growing for several months on artificial media:

Various stock cultures of bacteria.	Water.	11147.	11524.
17682	A.	B.	B.
17681	A.	B.	B.
11483c	A.	B.	A.
12004b	A.	X.	A.
9650d	A.	X.	A.
11604c	A.	X.	B.
9650b	A.	B.	B.
11604e	A.	X.	A.
11939e	B.	B.	A.
11483e	A.	B.	A.
9650e	A.	A.	A.
431'	B.	X.	X.
17688	A.	B.	A.
11939c	A.	B.	B.
17680	A.	B.	B.
11523b	A.	B.	A.
11939d	A.	X.	A.
11939f	A.	B.	A.

A = Good growth.

B = Poor growth.

X = No growth.

11147 and 11524 were amebas grown from dysenteric stools and were at the time of isolation very selective, being obtained with cultures of only one bacterium. Ameba "water," on the other hand, was isolated from the city water supply of Manila and showed very little selectiveness. Later, after being fed to monkeys, it became very much more so and was as difficult to reclaim from animal sources as any of those from the dysenteric intestine.

Special symbiotic bacteria.—The few observers who have grown amebas with a single microorganism have also found a variety of bacteria giving satisfactory symbiosis with their protozoa.

Beyerinck grew amebas with pure cultures of yeast as well as with a number of bacteria, including acetic bacteria and *B. coli*. Frosch was most successful with a nonsporebearing bacillus obtained from garden earth.

Tsujitani obtained successful results with *Spr. cholerae*, *B. typhosus*, *B. coli*, *B. fluorescens*, liq., *B. fluorescens non-liq.*, *Staphylococcus p. aureus*, *B. pyocyaneus*, *B. rubra*, and three varieties of bacilli from hay infusions. He failed to do so, however, with a bacillus from a liver abscess as well as with yeast and fungi.

Zaubitzer's most successful organisms were nonpathogenic ones. Mouton was successful with *Spr. cholerae*, *B. coli*, *Vib. metschnikoffi*, *Staph. p. aureus*, *B. anthracis*, *B. mallei*, and a yeast. Gottstein had the best results with bacteria obtained from garden earth.

Our own work has convinced us that the bacteria which under certain conditions produce satisfactory symbiosis include quite a large variety both of the pathogenic and of the nonpathogenic ones. Naturally, we have paid particular attention to the bacilli of the colon group, and it is with this class that we have had our most frequent successes in producing from the stools primary cultures which show the usual variations of the group. We have found that two organisms which are apparently identical culturally give different results as symbiotics for all amebas from a given source.

The first one with which we were successful was—

Bacillus 9650b.—Plates were made from stool 9650, and many colonies of all varieties of organisms contained therein were transferred to our media, and after three days all these cultures were inoculated from stool 11147 (dysentery). After forty-eight hours an examination showed that cultures made from colony *b* had many amebas, while those from the other ones were negative. This led to a more careful study of *Bacillus 9650b*. It appears to belong to the colon group, and morphologically and culturally does not appear to differ from others of the group. It is a rather short rod, slowly motile. It sometimes shows polar staining, while again it may be coccoid. It ferments glucose and lactose with the production of acid and gas. Litmus milk is quickly turned acid and coagulated by it.

Another organism which has proved very useful to us is *Bacillus 12935*. It is a yellow pigment-producing saprophyte, which may frequently be isolated from the city water supply or from plates exposed to the air. It is an organism which grows profusely, is motile, and measures about $0.5\ \mu$ by 1.5 to $2\ \mu$. In numerous animal experiments we have most frequently used this bacillus as a bacterium capable of symbiosis with amebas, because of the ease with which it may be recognized and reclaimed and because it is nonpathogenic for our animals as well as for man.

Bacillus violaceus Manila was isolated by Dr. P. G. Woolley, Director of the Serum Laboratory, from the lungs of carabaos and is described in Bulletin No. 15, Bureau of Government Laboratories. This bacillus has been very useful because of the great antipathy which some of our amebas have shown for it. Two of our cultures of amebas encysted when first placed with this organism, and apparently no further multiplication occurred. However, by gradually, during a period of two months, increasing the proportion of this bacillus we have succeeded in obtaining a satisfactory culture with no other organisms present. But even under these circumstances this culture required frequent transplants. On the other hand, we have amebas which from the first are able to exist symbiotically with this bacillus.

We have successfully used *Spr. cholerae* and several other varieties of vibrios, which have been isolated from various substances during the cholera epidemic in these Islands. Other bacteria which have acted more or less satisfactorily with some one or more of our amebas are *Staph. p. aureus*, *B. typhosus*, and many organisms not identified, from the air, water, liver abscess, and the normal and dysenteric intestine.

Distribution of amebas and sources from which they may be obtained by culture.—It is almost universally true that those who have worked on this subject at all seriously have succeeded in cultivating amebas from a great number of sources outside the human body. According to reports coming from all parts of the world, they have been isolated from soil (both surface and deep, 2 meters), prairie and mountains (1500 meters), marshes, slime, stagnant water, thermal springs; river, lake, and sea water; air, dust, dried grass, hay, fruits (both sound and decayed), including acid, decaying grapes, and many other substances.

In the majority of instances, particularly among the more recent workers, all cultivated amebas have been considered to belong to the so-called nonpathogenic type, and have usually been dismissed with this supposition without sufficient effort to determine their pathogenic significance.

From the nature of amebiasis and for other reasons, we are justified in believing that water and other substances are transmitters of the disease, and therefore must at times contain the infecting agent. With this belief we are justified in paying particular attention to the amebas from these sources; and since it has been proved, as we shall presently show, that some of these are dysentery producers, their extracorporeal distribution becomes of the greatest importance. We, as well as other authors, have demonstrated them in a large variety of materials; of this class we shall discuss in detail only those obtained from two important sources, namely, water and uncooked vegetables.

Amebas are found in large numbers in almost all of the surface waters of the Philippine Islands, and may almost constantly be cultivated therefrom without difficulty. This becomes all the more interesting when we consider the city water supply of Manila.

Amebas have been grown from every one of one hundred samples of water taken from hydrants in different parts of the city during the past six months, and with three of these cultures we have been

able to produce dysentery in monkeys. (Our culture "water" is from this source; it will be discussed in detail presently.) We have had no difficulty in cultivating amebas from the washings of vegetables, from a number of fruits, and from various other kinds of organic matter. Another of our cultures, "lettuce," was obtained from the fourth washing in distilled water of lettuce grown on the Government experimental farm in the city of Manila. Many surface soils in this country have been found to contain amebas in large numbers. We are now working with a number of cultures from soil taken at various distances from the surface.

In general, it may be said that the whole of the surface flora of the Philippine Islands carries a large number of these parasites. Some of which, at least, belong to the class which produces disease in human beings.

Distribution of amebas in animals other than man.—These protozoa undoubtedly have a wide distribution in the intestinal canal of many animals. They have been reported as present in the digestive tract of frogs, chickens, pigeons, lambs, calves, rabbits, dogs, horses, monkeys, and are probably existent in many other species.

Blanc found what he considered to be amebas in the lungs of a sheep. He says that the protozoa were larger than *Ameba coli*, had a single pseudopod, and produced in the lungs nodules resembling those seen in verminous pneumonia.

Generally no significance is given to the presence of amebas in the digestive tube of animals; they are thought by most authorities to be harmless. There are, however, a few observations which tend to show that this is not always the case, even when the amebas are ingested by natural means. Kartulis found them in the intestines of dogs which suffered from dysentery in Egypt. Cats have been reported to have the disease, and we have occasionally seen a spontaneous case in monkeys.

The question whether the passage of amebas through some of the larger animals increases their danger to man is one offering inducements for future work. It is just now particularly interesting to Manila because of the location and management of the new water supply. The present water sheds are inhabited by both human beings and animals; the new one will harbor only animals. The water from the present as well as the proposed new sheds contains large numbers of amebas. No practical means, on a large scale, has as yet been devised by which these protozoa may be removed from the water.

Distribution of amebas in man.—Amebas in several pathologic conditions have been found in man, both by culture and by microscopic examination. The more common sources, such as the intestine, the liver, etc., need not be mentioned here.

Miura found amebas in ascitic fluid from a woman suffering with abdominal tumor. They were present also in the bloody, mucous stools.

Celli and Fiocca cultivated amebas from the larynx in a case of tubercular laryngitis, ten times from the lungs in tuberculosis, six times in cases of pulmonitis, in fifteen cases of bronchial catarrh, three out of fifteen times from the female urinary tract, and once from the stomach of an infant. Amebas have been reported in scrapings of tartar from the teeth.

Kartulis isolated these microörganisms from a necrotic bone in the lower jaw. Flexner found them in an abscess located on the floor of the mouth and in a gangrenous surgical wound in a case of liver abscess.

Leyden and Schaudin encountered ameboid bodies in aspirated ascitic fluid in two cases of abdominal tumor. Baelz found them in the bladder and vagina of a young woman who later died of tuberculosis of the lungs and genitalia and who had hemorrhagic cystitis.

Jurgens repeatedly observed amebas in the urine of a patient, 58 years of age, suffering with tumor of the bladder. They were found by Wijnhoff in the urine of four patients.

Posner discovered amebas, some of which contained red blood cells, in the bloody urine of a man who had never been out of Berlin. This patient was suffering from a disease which, with recurrent attacks, had lasted for a period of a year. It was Posner's belief that the amebas had reached the pelves of the kidney.

The most important source of *cultures* of amebas in man is the supposedly healthy intestine, and to a less extent the dysenteric one. A number of observers have reported cultivating amebas from both these sources; but of these only a few state that they have grown the true dysenteric ameba, and their work has been severely criticised. Kartulis was one of the first to maintain that he had been successful in obtaining such cultures, and that he had reproduced the disease in cats by inoculation.

Our work in relation to this subject has progressed sufficiently to enable us to state that neither the physical condition of the patient nor the pathologic changes in the colon are the determining factors in the cultivation of parasites from the stool. Several of the amebas which we shall discuss in detail below were isolated from the dysenteric stools in cases of intestinal amebiasis (amebic dysentery), and another was grown from an amebic ulcer, the cultures being made at a fresh necropsy.

Cultural characteristics (general).—Cultures of amebas in general take on an appearance dependent upon the growth of symbiotic organisms, and so far as we have been able to see, there are

no appreciable differences between cultures from different sources when they are grown with the same organism.

So far as the parasites themselves are concerned, they do not seem to produce any microscopic evidence of their presence in the media, due probably to their transparency, and to the fact that they do not colonize or pile upon one another.

Another noticeable fact is that amebas do not develop below the surface in solid media. For this reason cultures are made in plates containing hardened media. This failure to develop to any extent in the depth is probably chiefly due to the density of the media and not to the partly anaerobic conditions which are present; for when the amebas happen to be below the surface of the medium in association with a liquefying organism, they may multiply, but do not extend beyond the liquefied area.

On the surface of plate cultures, where they may best be studied, the growth and spreading of most of the amebas, under favorable conditions, is quite rapid. By observing such a culture under the microscope, some idea is gained of the relation of the amebas to the bacteria, and the fact, of more or less importance, as to which precedes in culture, may be determined. In many instances the former travel quite rapidly and soon distance any bacteria excepting those directly in their track. Casual observations of plates may reveal them at some distance from other microorganisms. If, however, a very careful search is made, a few bacteria will be found to be following them very closely. Although the amebas may for a time take the lead, the process is not continued, for their movements soon begin to slacken until numerous bacteria have overtaken and even preceded them, whereupon their rapid progress once more begins. Division by fission may be frequently observed during this time. There is one very noticeable condition at this stage, namely, the general course of the amebas is away from the center of inoculation where the masses of bacteria are located, or to express it more exactly, they do not progress toward the bacterial masses. Amebas on the margin of the growth are practically always active, while the encysted forms are found nearer the center.

If one were to judge from the direct course which the amebas pursue away from the bacteria (fig. 3), it would appear as if they were trying to avoid the latter. Yet, as we have already observed, as soon as they are a short distance in advance, there is a slowing of motion until the bacteria again are in the lead, when the amebas

resume their former activity. This hide-and-seek phenomenon may be repeated by an ameba for hours, although eventually it begins to lag behind, and finally becomes round or encysted.

Morphological characteristics.—The measurements of amebas, as they are given by different authors, vary greatly, as the following brief list proves:

Marshall gives diameters as being from 10 to 40 μ , but usually 24 to 30 μ ; Fitcher, usually 12 to 26, the average being 20 μ ; Quincke and Rose 15 to 25 μ ; Kartulis, 12 to 30 μ ; Laffeur, 6 to 35 μ , usually 12 to 26 μ , varying much in different cases, but fairly uniform in the same stool; Osler, 10 to 20 μ ; Kruse and Pasquale, 10 to 30 μ ; Craig, 5 to 35 μ ; Zorn, 14 to 22 μ ; and Strong and Musgrave, 10 to 50 μ .

After further development of our work with cultures, and a determination of the life cycle, the morphology may become an important point in differentiating species, but at the present time no value can be attached to it. *It is certain that, with our present knowledge, the measurements of amebas from stools can not be used for purposes of differentiation either as to the species or as to pathogenicity.*¹

It is always easy to transfer amebas from a fluid to the surface of a solid medium, but the reverse of this process is not easily accomplished. Amebas isolated from water or some other fluid substance, after developing for a time on solid media, do not grow well, if at all, when returned directly to the fluid in which they formerly thrived. Amebas obtained from human sources are the most difficult to grow in liquids, and when they are first isolated from the intestine they do not do so at all.

In fluid media the greater proportion of the development at first takes place at or near the surface, but eventually it extends until amebas are found in small numbers throughout the substance.

¹ Morphologically, in cultures, amebas are also variable, the differences depending upon several conditions, such as the age of the parasites, the phase of the life cycle, the density of the media, and other factors of environment. In order to insure the greatest accuracy and to render them of any significance, the measurements should be made only when the animal is in the round stage, and preferably when encysted. Even when these precautions are taken, differences occurring in cultures from the dysenteric intestine will be found to lie between four and forty microns. Apparently there is considerable variation in the cysts grown from a single organism. The measurements of some of our amebas are given under the discussion of special amebas.

In a natural fluid environment, such as water, they are rarely found in the round or encysted stage, and they are but little more so in fluid cultures artificially prepared. When the conditions are suitable to life, encystment is apparently less frequent than when the amebas are placed in those which are unsatisfactory. Encystment may be brought about in a number of ways, for example, by a change in the reaction of the medium from neutral or slightly alkaline to slightly acid, by an increase in the amount of nutrition in the medium, causing the bacteria to multiply more profusely, by cold, heat, chemicals, etc.

Agglutination phenomena.—Agglutination phenomena have been noticed by Zaubitzer, who, with cholera vibrios, prepared cultures of his amebas grown on straw infusion. Serum from a guinea pig immunized against cholera was then added, and agglutination both of the vibrios and the amebas occurred. Neither the former nor the latter were killed by this process and could subsequently be grown by transplants. The author failed to find similar results when cultures with less toxic organisms were used, and he says that this was probably due to the fact that no satisfactory agglutinins could be obtained.

We have repeated Zaubitzer's experiments, and find as he did, that the agglutinins of a symbiotic bacterium have no destructive action on associated amebas. When the agglutination of the bacteria was brought about by a satisfactory serum all of the amebas did not encyst or become round, some of them continuing their ameboid motion without interruption.

Nutrition.—The means of nutrition of amebas are undetermined. Formerly it was supposed that the engulfing of certain substances within their protoplasm served this purpose, for amebas are known at times to take up red blood cells, bacteria, yeast cells, and other granular material. Recently the idea that these substances serve as food has failed of general belief. Apparently no one as yet has proved that the red blood cells are digested by the amebas, although these inclusions have been observed by different workers for varying lengths of time. Normal red cells have often been seen in their protoplasm, as have also bodies which were supposed to represent the remains of digested ones; but the process of digestion has never been followed out. On the other hand, amebas have been seen to discharge these cells, after the latter have remained within the protoplasm for some time, and these cells were

apparently still in a normal condition. In cultures the protozoa may often be seen to discharge all the granular material and foreign bodies which they contain just before entering the encysted stage.

Reaction of amebas to physical conditions.—The behavior of amebas towards varying physical conditions is important for several reasons, particularly as a knowledge of their influence will facilitate methods of classification and may throw new light on prophylaxis and treatment.

Drying.—Celli and Fiocca found that cultures of amebas would withstand drying for from eleven to fifteen months. Miller concluded that certain encysted amebas would withstand the same treatment for six years, and that others, particularly those which do not form well-marked cysts, were killed when subjected to this process.

Tsujitani, in order to obtain pure cultures from those mixed with the cholera bacillus, took advantage of the resistance of amebas to drying. Gottstein's cultures were killed on drying.

Zaubitzer's hay infusion ameba would resist drying for sixteen days, and would grow after complete drying when they were transplanted to culture media.

Our experiments on this subject are not completed, but it has already been found that there is a difference in the susceptibility of different amebas towards dessication; but we are not sure that this difference is an intrinsic one. Ameba 11524, after two months in plates which had during that time become dry and hard, grew on transplants; but Ameba 25624, and some others, failed in this respect even when transplanted to media inoculated with the original associated bacterium. We have some amebas which grow well on transplantation from cultures six months old, while others are apparently dead at the end of this time.

Temperature.—The influence of temperature on amebas merits careful consideration.

The optimum temperature for cultures, as reported by different authors, varies greatly, but as a rule it may be said to lie between 20° and 28° C. Some, however, have found 37° C. to be the most satisfactory. Gottstein gives the optimum at 20° to 22° C., the minimum at 10° C., and the maximum at 37° C.

All the amebas which we have so far studied, including those obtained from the intestine as well as from outside sources, flourish profusely at room temperature, but do not grow well at incubator temperature on the one hand and but very slowly at that of the ice box on the other. We have been unable to verify the

usual statement that amebas always lose their motility at or below 75° F. in stools, and certainly such is not the case in cultures.

As to the maximum temperature which amebas are able to resist, there appears to be some difference between the organisms described by the various authors.

Tsujitani says that cysts are killed by ten minutes' exposure to a temperature of 60° C., Celli and Fiocca consider 45° C. for five hours or 50° C. for one hour to be sufficient to kill them in the ameboid stage, whereas 60° C. for one hour does not destroy them in the encysted one.

Our results with reference to the maximum temperature have shown differences between amebas obtained from different sources.

EXPERIMENTS.

Ameba 11524 multiplied much more slowly in the incubator than at room temperature, and some reproduction occurred in the ice box.

Old encysted cultures of ameba water were exposed to 60° C. for one hour. Transplants to sterile media and to media containing the symbiotic bacteria of the original culture only, showed no growth. Controls produced large numbers of amebas in twenty-four hours.

Cultures of the same ameba, fifteen days old and in great part encysted, were exposed to 50° C. for one hour. Transplants developed no amebas, while the controls gave good growth.

Encysted cultures of ameba 11524, two months old, were exposed to 60° C. for one hour. Transplants to sterile media produced no growth; but in tubes smeared with the symbiotic bacteria of the original culture (*Spr. cholerae*) amebas developed in forty-eight hours. Cultures ten and fifteen days old and to a large extent encysted gave negative results on transplants after being exposed to the conditions above described. Cultures of this ameba one month old were exposed to 50° C. for one hour. Transplants to sterile media, as well as to media containing the symbiotic bacteria only, developed a good growth in forty-eight hours.

Cultures of ameba 11147, two months old, were exposed to 60° C. for one hour. Negative results were obtained when they were transplanted to sterile media as well as to media inoculated with the symbiotic bacteria of the original culture. Control transplants made just before heating developed a good growth in twenty-four hours. Cultures of the same ameba, one month old, were subjected to 50° C. for one hour with negative results on all transplants.

Nothing is said by those who have cultivated amebas with reference to the minimum temperature which the organisms are able to resist. Kruse and Pasquale, however, were able to produce dysentery in cats by using feces, containing amebas, which had been frozen and thawed; and from this they inferred that dysenteric amebas are probably not destroyed at a temperature somewhat

below 0° C. There are other observations in literature which indicate that at least some varieties of these parasites are very resistant to cold. The geographic distribution of dysentery, to say nothing of that of amebas in general, indicates that under certain conditions they are able to withstand intense cold. Our experiments with cultures have confirmed this, and have also shown that, as in the case of high temperatures, amebas are not all equally resistant under apparently similar conditions.

EXPERIMENTS.

Encysted cultures of Ameba 11524, two months old, were placed in cold storage at -12° C. for twenty-five days, and growth was obtained on transplant. With a fifteen-day-old culture subjected to similar conditions no amebas developed.

Finally, a culture of the same ameba, three and one-half months old, after being subjected to -12° C. for forty-five days, on transplant gave a growth of amebas, which, however, did not multiply very rapidly until the second generation. In one tube no growth was obtained on the second transplant.

The same experiments were repeated with Ameba 11147 and Ameba "water," but no growth was obtained on any of the transplants with either of these parasites.

Light.—The action of light on these protozoa is of interest because of its influence on certain members of the group, and it is particularly so at the present time when light therapy is attracting so much attention, and because the modern tendency is to explain the action of certain chemicals, such as quinine, on malarial parasites, by its fluorescent or other light effects.

Zaubitzer obtained growths of hay infusion amebas on making transplants from cultures which had been exposed to sunlight during eight to nine hours. However, if they were under similar conditions for an entire day no growth resulted.

Observations which show that red light causes amebas to stream and that light from the opposite end of the spectrum causes them to encyst are very old and have been often repeated. Dreyer has recently gone over this subject again with interesting results. He finds that encysted amebas are very much more resistant to light than those which are not in this condition. When the Finsen light was used the resistance was thirty to thirty-three times as great; with the use of light passed through uncolored glass five to six times; and with light passed through blue glass five times greater. He found that encysted amebas were destroyed in about twenty-five minutes by light passed through rock crystal (ultraviolet); in from sixty to seventy minutes by an equal quantity passed through uncolored glass, and in from seventy to eighty minutes by light transmitted through blue glass. According to his observations, the ultraviolet rays

are thirteen to fourteen times as powerful in their action as is white light, and eighteen to twenty times as much as blue light.

Schaudin proved that X-rays destroy certain amebas and other animal parasites, after they have been exposed to their influence for some time. Tappeiner and Jesionek demonstrated that fluorescent substances and daylight or sunlight destroy bacterial life as well as the properties of enzymes and toxins.

Dreyer found that the addition of a very small amount of erythrosine to media containing bacteria or infusoria render such organisms very much more susceptible to yellow and green rays. He recommended the application of this principle in light therapy by injecting 1 to 1,000 solution of erythrosine into diseased tissues before their treatment by application of Finsen rays.

Mouton has recently obtained some very interesting results, which in this connection are suggestive. He found that the intensifying of fluorescence in substance of the class mentioned, by the use of various rays, increases their action on certain cells, and suggested a therapeutic application of the phenomenon.

Our experiments with the action of light on amebas are not yet far enough advanced to justify very many definite statements.

Direct sunlight undoubtedly has at least an inhibiting influence on them. When fresh transplants of *Ameba* 11147 were exposed to sunlight for three hours no development occurred; but motile cultures of *Ameba* 11524, twenty-four hours old, were not killed by three hours' exposure to the sun's rays.

X-rays also exert an unfavorable influence on cultures. It was found that old encysted amebas would grow out on transplant after twenty minutes' exposure to this light. On the other hand, when young, growing cultures were exposed to it for twenty minutes, many rounded and encysted forms were observed and growth was frequently delayed on transplant.

However, the most interesting of the light phenomena is the action of fluorescence upon these protozoa, and we regret our inability to say more than the following about it at this time.

A young, growing slant culture of one of our amebas was placed inside a large tube containing a solution of fluorescein, so that the inner tube, which contained the culture, was entirely surrounded by the solution. This preparation, together with a control culture of the same age, was then placed in the direct sunlight for two hours. On microscopic examination all the amebas in the tube surrounded by fluorescein were found to be encysted. Transplants from the control tube gave a good growth, but those from the experimental one remained free from amebas.

Similar experiments have been made, using various fluorescent substances, such as quinine bisulphate, orcein, eosin, fluorescein, and resorcin, with different amebas as well as with growths of the same organism of different ages. Some of these substances, particularly quinine bisulphate, fluorescein, and orcein, under the conditions named, certainly, to a considerable extent, exerted an inhibiting influence on the amebas. This was also found to be true when the parasites surrounded by these solutions were exposed to the action of X-rays.

Theoretically at least, we should expect to secure the best results by exposure to ultraviolet rays concentrated through fluorescent substances; but the ultraviolet rays for experimental purposes are at the present time not available in the Philippine Islands.

Reactions to chemical agents.—In view of the therapeutic application of chemical agents, the reaction of amebas towards them is of the highest importance.

Perhaps the substance most generally mentioned in this connection is quinine, this being the one most universally used in the treatment of intestinal amebiasis (amebic dysentery). Lösch first noticed the parasitocidal action of the drug, and his observations have since been confirmed by several experimenters in so far as they refer to the action of quinine on amebas in the stool. The dilution supposed to have the most efficient action is variously given, the proportions ranging from 1 to 300 to 1 to 1,000. Some authors have expressed doubt as to whether quinine possesses any specific action.

EXPERIMENTS.

A slant culture of Ameba 11147, twenty-four hours old, was washed with a 1 to 2,500 solution of quinine hydrochlorate. The amebas quickly encysted, and in from five to eight minutes many of them had broken up and disappeared. Ten minutes afterwards no development of amebas occurred in cultures from this emulsion, although the bacteria showed satisfactory multiplication.

This experiment was repeated with similar results with Ameba 11524. However, a scant growth of amebas was obtained when transplants from the emulsion were made ten minutes afterwards.

Forty-eight-hour-old plates of Amebas 11147 and 11524 were exposed for ten minutes to formalin vapor. Transplants failed to develop amebas.

Encysted cultures of Ameba 11524, two months old, were washed off with a 1 to 1,000 solution of formalin. The parasites were quickly encysted; but in twenty-four hours a small number of amebas developed on transplants which had been made from the emulsion five minutes after exposure. When cultures of Ameba 11147 of the same age and under similar conditions were used, identical results were obtained; but when Ameba "water" was employed, no growth was obtained on any culture.

Old and young living cultures of Ameba 11524 were washed off with carefully neutralized solutions of acetozone of 1 to 1,000 to 1 to 10,000. In some instances the parasites were encysted; but, even when the 1 to 1,000 solution was used, they grew out on transplants made after five minutes.

With the use of carefully prepared solutions of acetozone, 1 per cent acid to phenolphthalein, the findings were entirely different. This slightly acid solution produced results which were most satisfactory and out of all proportion to any difference attributable to the small amount of acid present, which of itself would exert but a very inconsiderable inhibiting influence upon the protozoa.¹

EXPERIMENT.

Ameba 11524, in cultures of all ages, from those of young motile ones to those encysted for six and one-half months, have been washed with solutions of 1 to 5,000 to 1 to 2,000 of this slightly acid acetozone, and in every case have given negative results on transplant (after ten minutes) to sterile media as well as to media smeared with the satisfactory symbiotic bacteria.

A phenomenal resistance to alkalies has been noticed by several observers. All our cultures, whether young or old, motile or encysted, were killed by 20 per cent solutions of sodium hydroxide or potassium hydroxide, as demonstrated by their failure to develop on transplants made after five to fifteen minutes.

EXPERIMENT.

Ameba 11524 was transferred to media with varying amounts of sodium hydroxide and potassium hydroxide in order to determine the maxi-

¹ There may be two explanations of the difference between the action of benzoyl-acetyl peroxide (acetozone) in alkaline or neutral solution and in acid. The first is that, if the solution is alkaline, it is not improbable that the acetoperacid, produced by the hydrolysis of benzoyl-acetyl peroxide, either liberates a minute quantity of chlorine from the chlorions or, as seems more likely, is able to oxidize the chlorious to hypochlorite, the latter having an energetic oxidizing action. The second would be that acetoperacid, or benzoperacid are much more rapidly decomposed, with the formation of hydroperoxide and the salts of acetic and benzoic acids, in alkaline than they are in acid solution. However, hydroperoxide has very feeble germicidal properties and hence, probably also a feeble action on protozoa, whereas the per-acids are intensely germicidal. Therefore in the great dilutions which are used, the alkali might very readily so hydrolyze the per-acid that it would practically be inert, whereas its full effect would still be present in the acid solution.—FREER.

imum degree of alkalinity in which they would propagate. 1 to 5 cubic centimeters of N/10 solution of these chemicals were added to tubes containing 10 cubic centimeters of media neutral to phenolphthalein. We were unable to secure a good growth on cultures which contained more than 3 cubic centimeters of the alkali.

However, what is of more importance, from a practical standpoint, is the proportion of acid which may be present and still allow these parasites to propagate. They are very susceptible to the action of acids, but, as we have determined, some of them multiply in media which are more acid than is usual in the normal stomach.

Ameba 11524 grew in a medium $\frac{1}{10}$ per cent acid (HCl) to phenolphthalein. By starting with a neutral medium and a symbiotic organism, like *B. coli*, which multiplies well in acid media, and by gradually increasing the acidity on successive transplants, multiplication may be obtained on a medium even more strongly acid than this.

Sera, blood toxins, etc.—An understanding of the action of these substances is essential to a proper knowledge of the infection produced by amebas, and we have therefore begun some preliminary work on this topic.

Emulsions from Ameba 11524 in a pure culture of *Spr. Cholerae* were treated in the hanging drop with equal parts of a serum with a high agglutinating power for the latter organism. The bacteria were very promptly agglutinated, and some of the amebas assumed a round form after a few minutes. However, many of them remained motile for one hour and grew on transplant at the end of this time.

A dog was given frequent subcutaneous and intraperitoneal injections of a culture of Ameba 11147 and *B. coli*. At the end of two weeks this animal's serum had no parasitocidal action on amebas, which developed when transplanted to a plate the surface of which was smeared with its serum.

A monkey was given in the abdominal cavity, on every fourth day, injections of Ameba 11147, together with a yellow pigment-producing bacillus. Five days after the last injection the serum of the monkey was found to have no unfavorable action on amebas in the hanging drop, and transplants developed on plates smeared with it. There was no variation from these results when the experiment was repeated with Ameba 11524.

When a few drops of human blood or a small amount of human serum were added to fluid cultures of *Ameba* "water" of the first few generations, the parasites all encysted in a very few minutes and refused to grow when transferred to plates on which the surface of the medium had been smeared with human serum. When similar cultures of these amebas were injected subcutaneously or into the liver of a rabbit or monkey, the protozoa which were reclaimed by culture from the abscesses were not so susceptible to the action of human blood or serum as they had been. When cultures of amebas from straw infusions were used, the results were similar to the foregoing; but with those of *Ameba* 11524 no apparent effect was produced by the introduction of human serum and good growth was obtained on transplant. This demonstrates the influence of animal tissues upon the adaptability of these parasites, a property which can still further be verified by cultures.

If to cultures of *Ameba* "water" very minute quantities of serum or blood held in solution by potassium citrate are added, and the amounts are gradually increased on successive transplants, a culture may eventually be obtained, which flourishes notwithstanding the presence of a considerable quantity of blood or serum. The addition of the blood, however, enriches the media to such an extent that the bacteria develop very rapidly, and to avoid this it is advisable to make alternate transplants to those free from blood.

Cultivation of special amebas.—We purpose here to discuss briefly, under their laboratory numbers, a few of the special amebas which we have cultivated. In order to show conclusively that we are dealing with amebas which cause dysentery, a brief description of the clinical manifestations of the cases is also introduced.

Ameba 11524 was isolated from a dysenteric stool. The patient, an American nurse, had been suffering with intestinal amebiasis (amebic dysentery) for about one year and amebas had repeatedly been found in her stools during that time. Her treatment was of an intermittent character, being suspended with the subsidence of clinical symptoms. The course of the disease was the usual one, with very chronic tendencies and with frequent and sometimes quite severe exacerbations.

Our first cultures were made during such an exacerbation, and at a time when there could be no reasonable doubt as to the correctness of the diagnosis. From a specimen containing many motile amebas, from 20 to 35 μ in diameter, and carefully taken in a

manner which prevented outside contamination, a large number of cultures were made as follows. Fifteen sterile Petri dishes were charged with our special medium, which was then allowed to harden. The surfaces of twelve of these plates were then smeared with pure cultures of different bacteria from stock cultures, including those cultivated from the intestine in other cases of amebiasis. All the plates (including the three sterile ones) were inoculated with the stool containing the amebas by merely smearing small quantities over the surface of the plates with a platinum loop.

These cultures were examined at the end of twenty-four hours and no amebas were found; but on a second examination, forty-eight hours after inoculation, two out of the fifteen contained large numbers of motile protozoa. These two cultures were identical, being symbiotic with a bacillus (9650b) which has already been described. Examinations made as late as seventy-two hours after inoculation revealed no amebas in the other cultures.

Transplants with the first, second, and third generations, as in the first case, were successful only with media which had been smeared with *Bacillus* 9650b. Transplants from the original culture made on the fifth day failed to grow even under these conditions; those from the second generation after the same interval showed a growth, but none occurred on those made on the tenth day. The fourth and subsequent generations developed by transplanting to sterile media, and only those bacteria which were carried over in the loop during the transfer were present.

Experiments with this ameba on other media have been unsuccessful. At best but a scanty growth has been obtained upon somatose agar, and reproduction on transfer to various fluid media has been scarcely more satisfactory. We have, however, by gradual reductions in the density of the substrata, succeeded in obtaining good growth in fluid media composed of beef extract 0.5, salt 0.5, water 1,000, made with a reaction 1 per cent alkaline to phenolphthalein.

Transplants to old cultures of stock bacteria grown on ordinary laboratory agar have not given satisfactory results. On such cultures encysted amebas could be found among the bacteria for several days, but there was little or no evidence of multiplication. Five months after this ameba had been isolated and had become well accustomed to artificial media, it gave growth when transplanted from a culture with comparatively few bacteria to a

sterile ordinary laboratory agar slant; but as the bacteria on this medium increased, the amebas which were transferred were soon lost.

Growth was found to be very satisfactory for a long time on a medium composed of 2 per cent agar and 0.5 per cent of beef extract (1 per cent alkaline), the development decreasing only when a marked diminution in the number of bacteria, which is usual with this medium, occurred.

Microscopically this protozoon, as obtained from culture, is indistinguishable from those seen in the stools of the patient, and it is a true dysenteric ameba. Its measurements in the round stage in the stool were 25 μ to 35 μ , and those in the cultures generally corresponded with these figures, but they varied greatly, owing, no doubt, to environment and the phase of the life cycle at the time of the examination.

Encystment of the ameba, when it was in unsatisfactory environment, was very rapid, and when once this stage was reached, no further reproduction could be observed until it was transplanted. On suitable media multiplication by fission was frequently observed, and motile amebas often remained present in the cultures for days. However, the final result always was encystment, which began at the point of inoculation and progressed toward the outer margins of the spreading growth, where the youngest parasites were the last to undergo the change.

A very interesting feature of these amebas is the variation in the character of the pseudopodia, which ranges from a decided lobose to the most exquisite spinose. These differences, at least in part, depend upon physical environment.

In our collection there now are cultures of this ameba which were started from a single parasite. They are in pure culture with four different bacteria—*B. coli*, *Spr. cholerae* and two different pigment-producing saprophytes. The protozoa grows well with all these organisms, and by methods already given has been changed from one to the other and *vice versa*.

In one instance, dysentery in man followed the ingestion of three gelatin capsules filled with scrapings from the surface of cultures of this ameba in symbiosis with a harmless bacterium. Dysentery has also been produced in monkeys by similar cultures as well as with others where the bacterium in symbiosis was a pathogenic one.

Ameba 11147 was isolated from the stool of a male Filipino,

who had suffered from recurrent attacks of intestinal amebiasis for about five months, and who had apparently recovered under treatment. The diagnosis of intestinal amebiasis was confirmed by post-mortem examination three months later.

The technique used in isolating this ameba was identical with that described for Ameba 11524, and the only growth obtained was on plates which had been smeared with the same bacillus as in the first case.

The second generation of this organism grew on sterile plates, to which only those bacteria which were carried over in making the transplants were added; and no further trouble has since then been experienced in keeping satisfactory cultures. On three different occasions cultures were obtained from single ameba. No differences were apparent in these three cultures, and we therefore concluded that the original one was of a single species.

Cultures from a single ameba were then transferred to pure ones of several bacteria, including *B. coli*, *Spr. cholerae*, and two pigment-producing saprophytes. The growth with all these organisms was good, after it had once been obtained. The symbiosis of this ameba has been changed from *Spr. cholerae* to a yellow-pigment-producing saprophyte, obtained by exposing plates to the air and back to *Spr. cholerae* on two different occasions.

Attempts to cultivate this organism on other media gave results very similar to those already described for Ameba 11524. As with the former and all others, an examination of cultures will serve to distinguish the unsatisfactory media, in which the amebas encyst in a very few hours, with no further development until they are transplanted to a more favorable environment; whereas, in suitable media multiplication continues for days before encystment (the final result in all cultures). When the latter stage is once reached, no further development may be expected until transplants are made. These are successful in the interval from one week to at least seven months.

The longevity of the amebas, not considering the probable differences in species and the length of time during which they have been kept on artificial media, is apparently influenced to a certain degree by the class of microorganisms present in symbiosis; for while all transplants from old cultures with one bacterium may give good results, those from similar cultures with another bacterium may be negative.

Ameba 11147 is apparently pathogenic. In the stools is measures from 22 μ to 28 μ , its average in culture being about the same. As has been found true of all our cultures, however, there is considerable variation in size, depending partly no doubt upon environment and partly upon phases of development. The pseudopodia thrown out by this organism in culture are of such a character as to place it in the lobose variety; but under certain conditions, not yet fully understood, but in all probability depending upon the density, viscosity, and other physical properties of the media, they may be of another type, including the spinose. In cultures this ameba has been repeatedly observed to multiply by fission, and it apparently has distinctive characteristics. Dysentery has been produced in monkeys by feeding old cultures of it in symbiosis with both pathogenic and nonpathogenic bacteria.

Ameba "water" was isolated from the water supply of Manila, from which, during a considerable portion of the year, these parasites may be obtained almost at will. We have made numerous cultures of amebas taken from taps in the city and from the Mariquina River, the source of the water supply, at various places along its course from the origin to the intake. However, in this report we shall consider only one individual, which was isolated about a year ago and has been cultivated on artificial media since that time.

The culture was started in two days. First, some water was centrifuged and the deposit from the bottom of the tubes was transferred to sterile plates prepared as already described; amebas developed at the end of twenty-four hours. By the other method, which is the one which we now use in our routine work for the cultivation of amebas from water, or other outside sources, 200 cubic centimeters of water was drawn into a sterile flask, to which was added 0.5 to 1 cubic centimeter of ordinary laboratory bouillon (1 per cent alkaline to phenolphthaleïn) for each 100 cubic centimeters of the sample. After from twenty-four to seventy-two hours amebas may be found on the surface of the fluid, and with a platinum loop transferred to plates.

This ameba, as is true of all our water amebas, grew well from the beginning without the addition of bacteria other than those associated with it. At the time of the transfer of this organism from the fluid to the solid medium, it was associated with several other varieties of parasites, mostly *ciliata*, some of which endured through one or two transplants on solid media, but at length

entirely disappeared. For some time after we began its cultivation, it grew readily on a variety of fluid media, including most of those recommended by previous experimenters; but after some months on solid media it became much more selective, and it is now quite difficult to transplant it directly to any fluid. Shortly after its isolation a pure culture—so far as the amebas are concerned—was secured by the methods already discussed. Later it was grown with pure cultures of several organisms—the “pure mixed culture” of Frosch.

In its behavior on other media this parasite resembles the two already described. Of all our amebas which form true cysts, this one is the most susceptible to the action of a luxuriant growth of bacteria. When placed in suitable medium and under proper conditions of symbiosis, it lives for a long time; but with an unsatisfactory bacterium, transplants may not grow for two weeks. We have succeeded in transplanting it from favorable tubes after five months, and again we have entirely failed to note a growth from cultures one month old.

Morphologically this ameba closely resembles *Ameba* 11524, but it apparently has certain differences. Its size varies considerably, evidently under the influence of certain physical conditions as well as of others which we do not as yet fully understand. Full grown amebas at rest or encysted measure from 15 μ to 35 μ .

This ameba we believe to be capable of causing dysentery, for we have produced a disease which is clinically and anatomically identical with it by feeding old cultures to monkeys.

Ameba “lettuce” was isolated from a head of lettuce grown on the agricultural experimental farm in Manila. The technique used in its cultivation closely resembled the one employed for water amebas. The lettuce was shaken with water in a sterile flask. Afterwards the water was decanted into other sterile ones, and the washings repeated four times. Amebas were present in all of the cultures from the decanted fluid. As was true of water amebas, the organisms from lettuce grew from the beginning without the addition of special bacteria, and cultures have been continued on solid media during a period of five months. From a single ameba, cultures have been obtained with only one variety of bacteria. The lettuce ameba is somewhat more restricted in its selection of symbiotic organisms than are some of the others. In size it shows great variation and its cyst formation is of an indistinct type. Favorable

cultures of this ameba were fed to three monkeys, and amebic dysentery was produced in two cases.

Ameba 25624 was isolated from the stool of a Filipino, who gave a history of intermittent diarrhea with occasional exacerbations covering an interval of three months. Mucus and blood were present in the stool during such times. The patient's evacuations at the time of the first examination were semiliquid and contained a considerable amount of mucus with a fair number of red blood cells. Two careful examinations, however, failed to demonstrate the presence of amebas.

Cultures from one of the specimens, the precautions being the same as with *Ameba 11524*, gave positive results on two plates—one of sterile media inoculated directly from the stool and the other previously smeared with a pure culture of the yellow pigment-producing saprophyte described under *Bacillus 12935*. The plates which had been inoculated with *Bacillus 9650b*, which it will be remembered was the satisfactory organism for both *Ameba 11524* and *Ameba 11147*, gave negative results. No difficulty has been encountered in keeping this ameba alive on transplants, and it is now one of our stock cultures.

This is one of the most interesting parasites which we have cultivated, and in a general way it closely resembles ameba lettuce. More care is required in its propagation, as it is particularly selective as regards symbiotic bacteria. In comparison with some of the others, it will not remain alive as long without transplanting, and in many ways it shows itself to be a more delicate organism. It has been seen to multiply by fission on plates, but it is certain that another method of division exists. Judging from this as well as from its condition when at rest, we feel sure that it is a distinct variety and also that it is an ameba capable of producing dysentery.

Ameba "C" was isolated from the stool of a native, who at the time we secured the culture denied having had dysentery or diarrhea. This patient, together with a number of others, all of them with negative histories of diarrhea or dysentery, passed stools which on examination contained fair numbers of amebas after a cathartic dose of Carlsbad salts. At the time of examination the patient in question was in the hospital because of a fracture of the leg, and his bowels remained normal for eighteen days, when diarrhea developed and with the usual intermissions soon reached

a stage where a diagnosis of intestinal amebiasis was justifiable on purely clinical grounds.

The methods used for the culture of this ameba were the same as those already described for the other intestinal amebas. They did not develop on any of the sterile plates inoculated directly from the stool, and only two of those previously smeared with bacteria gave a growth. In all its cultural manifestations this ameba closely resembles *Ameba* 11147, which has already been described.

Ameba 34354 was obtained from the stool of a patient suffering from dysentery. In this case the patient died and the same ameba was grown post-mortem from ulcers of the colon. The case was that of a private patient of Dr. Strong, to whom we are indebted for the clinical and post-mortem data used. The patient was an American, whose dysentery, at the time of his death, was of about seven months' duration, treatment having been uninterrupted during that time.

Some three months before death we completely failed to grow amebas from a specimen of his stool, though microscopically it contained large numbers of these parasites embedded in bloody mucus; and on the day before death another attempt was made. A large number of plates were inoculated, amebas being obtained from three; one of these was a sterile one, inoculated directly from the stool, and the other two had been previously smeared with bacteria which had formed satisfactory symbiosis with other amebas.

At the necropsy we made a large number of cultures from the ulcers of the colon caused by amebas and from two of the several liver abscesses. For the intestinal ulcers amebas were successfully grown, but not from the liver abscesses.

No discussion of this organism will be given at the present time, as it has been too recently isolated.

Ameba 17786 was isolated from the stool of a patient who had probably suffered from amebic dysentery during several months, but who had developed clinical symptoms for thirteen days only. Plates, with and without bacteria, were made in large numbers, the organism developing only on one which was sterile and inoculated directly from the stool.

Ameba 12485 was cultivated from the stool of a member of the Laboratory staff who had been in the Philippine Islands five months, but the diarrhea was of only two days' duration at the time the cultures were made.

The plates were made both with and without bacteria in the usual way, and a satisfactory development of amebas was secured in several, including both those free from special bacteria and those to which such organisms had been added.

Notwithstanding treatment was promptly instituted, the patient continued to have exacerbations, and at the present time, six months after the first cultures were made, still has decided symptoms of dysentery.

On two subsequent occasions we have failed to cultivate, on sterile media, amebas from specimens of this patient's stool. Experiences similar to those in this case have also been encountered in others often enough to justify the conclusion that *as a rule* the earlier the stage of the infection, the oftener will ameba grow when specimens of feces containing them are inoculated directly to sterile media.

Ameba 12483 was grown from the stool of an American who had been suffering with intestinal amebiasis for about six months. At the first attempt no amebas were obtained, although a large number of plates were used and inoculations were made directly from the stools upon sterile media and upon that previously smeared with a number of bacteria which had proved satisfactorily symbiotic with other amebas. After this failure, agar-plate cultures were made from the stool, all the colonies which grew were isolated in pure cultures, and the amebas were successfully cultivated on media smeared with some of these bacteria. No growth occurred on any of the controls, which were prepared as in the first instance. On working out the bacteria, whose symbiosis furnished the positive results, it was found that they were all organisms of the colon group, and apparently identical. These amebas were quite selective for this bacillus for several generations, but later could be satisfactorily grown with a number of organisms. In its general cultural characteristics it closely resembles *Ameba 11147*.

Ameba "1" was grown from the dysenteric ulcers of a patient who had not shown clinical evidence of the disease during life, and who died of an intercurrent lobar pneumonia. This patient, a native, was an inmate of Bilibid Prison, and upon admission to the hospital amebas were found in his stools, although he had no clinical symptoms of dysentery and failed to give a history of previous attacks. Up to the time of his death, nine days after admission, no bowel trouble had developed. At necropsy, which was performed three

hours after death, the classical lesions were found in the cecum and the ascending colon, and to a less extent about the splenic flexure. Made with the usual technique, cultures from the floors of the ulcers gave amebas on two plates which had been smeared with *B. coli*.

Ameba "L" was cultivated from the stool of a patient on the second day of the disease, as determined by clinical symptoms and history. It grew well from the first, both on sterile media which had been inoculated directly from the stool as well as on that in which *B. coli* had been used as a symbiotic organism.

Ameba "G" was grown from the stool of a young lady who at the time was not suffering from diarrhea and gave no history of such trouble. The culture was made on the day following a rupture into the bowel of an ischio-rectal abscess. From that time the patient developed clinical symptoms of amebiasis with the presence of amebas in the intestinal contents.

The amebas grown from this case are slightly below the average size, although in the round stage on favorable cultures some of them measure 25 μ . They are not as viable, and, as do most of our cultures, require frequent transplantation.

Ameba "urine" was grown from urine drawn aseptically from the bladder of a man suffering from hemorrhagic cystitis. This case was under the care of Dr. J. R. McDill, and will be reported fully by him at a later date.

Further work may, and probably will, show that some of the amebas which we have described are identical, and for this reason we have avoided naming or individualizing them further than by attaching their laboratory numbers for convenience in discussion.

On the other hand, judging from what has already been learned of them, there would seem to be more than one variety. When we remember that two of the most important ones which show particular differences are from the amebic colon and that the third is from the city water supply, the multiplicity of dysentery-producing amebas is, to say the least, very strongly indicated. If this is fully determined to be the case, our nomenclature will need some revision, and the first ameba shown to comply with Koch's laws should retain the old name *Ameba coli*.

III. ETIOLOGIC SIGNIFICANCE.

The etiologic importance of the amebas in the intestinal and other infections with which they have been found associated has

been a most debated question almost since the time of the appearance of Lösch's report in 1875. For some time after the publication of his article the literature was meager, but those who reported finding of amebas in dysenteric stools considered them, as Lösch believed them to be, the cause of the disease. The work of Cunningham, Grassi, and others, reporting the discovery of amebas in the stools of healthy persons and of those suffering from other diseases, led to the conclusion by some writers, that these protozoa probably have nothing to do with the production of dysentery. The discussion has never been fully settled. The literature on the subject is too voluminous to review, but it may be briefly discussed under the four headings given below, before taking up the results of our own work, which shows that amebas are the etiologic factor in this form of dysentery.

- (1) Amebas are harmless commensals.
- (2) They intensify or alter the lesions already present.
- (3) There are pathogenic and nonpathogenic amebas.
- (4) All amebas are, or may become, pathogenic.

The first of these propositions has had many supporters, from Cunningham in 1881 to Duncan in 1902, the arguments advanced having varied but little. The most important, and the one almost always brought forward, is that amebas have been found in healthy persons. The evidence of the truth of this assertion is based upon a large number of observations, chiefly those of Grassi (1879); Lewis and Cunningham (1881, during a cholera epidemic in India); Calandrucio, Kruse and Pasquale in Italy, the latter authors finding amebas in their own normal stools and in those of twenty healthy persons out of a total number of thirty-five examined; Schuberg (1893), who, after the administration of Carlsbad salts, found amebas in the stools of about ten out of twenty healthy persons; Celli and Fiocca (1895), who isolated amebas from the intestinal canal of two out of fourteen healthy infants, and from three boys in Alexandria after the use of Carlsbad salts (they also cultivated *Ameba coli* from the stool of a healthy infant); Fiori (1896), who encountered encysted amebas in normal stools and motile ones after the administration of salts; Ciechowski and Nowak (1898); Strong and Musgrave (1900), who found amebas in 4 per cent of a series of specimens obtained after the administration of Rochelle salts to soldiers not suffering from dysentery or diarrhea; Huber (1903), who encountered them in about twenty per cent of the cases examined; while, on the other hand, Janowski, Dock, Zorn, and many others have failed to find amebas in the healthy intestine.

When critically reviewing literature, but little of it seems to be conclusive. All the positive observations except part of those of Celli and Fiocca and one of Strong and Musgrave have been

determined from single examinations, without being confirmed by the subsequent histories of the patients, and from what we know now of the nature of the disease, they are of but little value. Celli and Fiocca, in reporting the presence of amebas in three boys in Alexandria, say that there was no history of subsequent bowel trouble, but they do not give the period during which these observations were continued. One of Strong and Musgrave's patients remained well for about three months, at the end of which time he passed from observation. This period, as we shall show below, was not of sufficient length to prove either that the bowel was healthy or that the amebas were harmless in this case.

The statement of Kruse and Pasquale that amebas were found in their own normal stools probably proves nothing more than that these parasites may be transiently present in the normal bowel, unless they made repeated observations over a longer period of time or did not subsequently suffer from intestinal trouble. On this subject their article makes no statement.

It is thus seen that there are but few authentic accounts of even the transient appearance of amebas in the stools of supposedly healthy persons. Post-mortem examinations have given no different result, because, so far as we have been able to determine, no records of amebas being found post-mortem in a normal intestine in cases in which during life amebas have persisted for any length of time, or indeed have propagated in the intestine.

Finding amebas at necropsy in the intestine without microscopic lesions is not conclusive evidence that such amebas were harmless, even though the parasites were shown to be present in the stools during life for a time less than the maximum period of incubation, which may be more than five months. Such observations, if made without a study of microscopic sections, are still further open to criticism, for there is a preulceration stage in the infection. Owing to an epidemic of pneumonia among the inmates of Bilibid Prison, where amebiasis is also quite prevalent, we have been able to study these early lesions quite extensively, and the findings have fully justified our criticisms of reports which pronounce an intestine containing amebas healthy without a microscopic examination of sections. *We do not wish to state that amebas are never present in the normal colon, for such may occasionally be the case;* but, after considering what has been said above, one is justified in saying that even their transient presence

in the normal human bowel is a much less frequent occurrence than it is generally stated to be by authors, and that the evidence brought forward in support of the conclusion is not free from criticism.

Whatever may be said of the transient presence of amebas in the normal intestine, their harmless persistence and proliferation there for a time equal to the greatest known incubation period of the disease has not been demonstrated. The observations of Celli and Fiocca in the case of three boys, and the one of Strong and Musgrave, which has already been mentioned, do not fulfill the requirements, and are therefore not conclusive.

To illustrate the incubation period in this infection and in general to see how far the arguments submitted above may be substantiated, the following is offered:

Large motile amebas were found in the stool of a 3-year-old boy, who gave no history of past or present diarrhea and who was apparently in good health at the time of examination. Amebas continued in the stools and the boy remained in good health until the one hundred and eighteenth day after the first examination, when a slight diarrhea developed, and with the usual intermissions progressed to the true clinical dysentery; and he was placed under treatment on the one hundred and twenty-fifth day after the amebas were first found.

A native boy, 18 years old, came under observation for fracture. His stools contained fair numbers of amebas, but there was no history of diarrhea and his bowels remained normal for twenty-two days after admission to the hospital. On the twenty-third day slight diarrhea developed and continued for two days. Shortly after this he contracted plague and died, and at the post-mortem examination the colon showed typical lesions of amebiasis which must have existed for months.

We repeatedly found amebas in the stools of a man in very good health during a period of nearly five months. During this time there had been a few slight attacks of "indigestion," some loss in weight, and occasionally slight indefinite abdominal pains, but no diarrhea. During the sixth month diarrhea developed and gradually became more severe, until the diagnosis of amebiasis was fully borne out by the symptoms. He is still under treatment.

Amebas were found in the stools of five out of forty apparently healthy natives after the administration of Carlsbad salts. One of the positive cases was lost sight of; three of the others were undoubtedly suffering with amebic dysentery at the time of the examination, a fact which became more evident as the disease progressed, because blood and mucus finally appeared in their stools, so that they ultimately were placed under treatment. The remaining individual continued to have normal passages and developed no symptoms until the eighteenth day after the first examination, when he suffered with diarrhea for two days, the dejecta containing

numerous amebas but no blood. He had no further trouble until the twenty-sixth day, when there was a second light attack of diarrhea lasting about the same length of time, amebas as well as a few blood cells still being present. After this the attacks of diarrhea became more frequent and severe, the case gradually merging into a condition which left no doubt of the diagnosis of intestinal amebiasis.

The stools of 300 miscellaneous prisoners in Bilibid Prison were examined after the administration of Rochelle salts and amebas were found in 101 of them. Sixty-one of the positive ones were suffering with dysentery, but the other 40 gave no history of past or present diarrhea.¹

During the next two months 8 of the latter died of intercurrent diseases, and satisfactory evidence of amebic infection was present in all. A second examination of the remaining 32 made two months after the first showed 26 positive and 6 negative for amebas, and 15 of these were suffering from dysentery.

Thus during two months of observation of 40 patients who had amebas in their stools without symptoms of dysentery, 8 died with lesions of the infection, 15 others developed dysentery, and the remaining 17 are still under observation.²

Finally, if we admit, a point unproved as yet, that amebas may be transiently present in the normal colon or even that they may propagate there, it is not proof that amebas are harmless. Such a conclusion would be equally applicable to others of the infectious diseases with generally recognized etiology; it leaves natural immunity and other factors out of consideration, and most important of all takes no notice of the influences which environment may have on the parasites themselves.

Clinical observation furnishes good evidence that all these factors play a part in amebic as in other infections. For example, the disease in children and natives is much less severe and more amenable to treatment than in adult foreigners, and in the former we have seen more than one instance of recovery without treatment. The influence of the environment of the amebas with reference to their pathogenic action has already been mentioned in another part of this paper.

¹The average native's statements are useless for purposes of clinical diagnosis, and particularly so with reference to intestinal troubles, from one to five stools in twenty-four hours being considered normal by many of them.

²In a third examination made six weeks after the above, 2 of the 17 patients had been discharged from the prison and the remaining 15 were positive for amebas, and clinically the diagnosis of dysentery was perfectly apparent in all of them.

The next most important argument generally submitted that amebas are harmless commensals is that they have been reported in the intestinal contents of persons suffering with diseases other than dysentery.

Such reports have been rendered by Cunningham, who found amebas in 18 per cent of the stools of cholera patients in India; Celli and Fiocca, in the intestinal contents of twenty-four out of sixty-four infants afflicted with intestinal diseases; Ciechanowski and Nowak in tubercular ulcers of the intestine; Berndt (1894), in subphrenic abscesses following typhoid fever; Gasser (1895), in stools of five out of eight patients suffering with chronic diarrhea following dysentery; Normand, in colitis; Bizzozero, in chronic proctitis; Massiutin, in typhoid, pallegria, acute and chronic intestinal catarrh, chronic diarrhea, colitis following tumors of the colon; Grassi, in diarrheic and dysenteric intestinal diseases; Perroncito, in chronic enteritis, complicated by diarrhea; Babes and Zigura (1895), in fifteen cases of entero-hepatitis; Borchardt (1896), in stools of a patient who had been suffering with intermittent diarrhea for six years; Casagrandi and Barbagallo, in several other diseases than dysentery; Kruse and Pasquale; Quinke and Rose; Schuberg and others.

In looking over this literature one is struck with the fact that nearly all of the diseases mentioned above involve the colon—indeed, the majority as diagnosed are principally of this organ, and further with the fact that most of the observations were made in places where amebic dysentery was undoubtedly endemic at the time.

We have given particular attention to this subject for a number of years in a country where amebiasis is endemic; and while we have repeatedly found amebiasis associated with a variety of other diseases, we have not found in such observations evidence of the harmlessness of amebas. For example, during the late epidemic of cholera in this country amebas have been found in the dejecta of cholera patients, and in some of these cases which were examined post-mortem, the lesions of an old amebic dysentery were found in addition to those of cholera.

The same may be said of typhoid fever. We have complete records of a number of cases of typhoid fever and amebic dysentery in the same patient, in some of which amebas were found in the stools during life, and the diagnosis of a double infection confirmed at necropsy.

With reference to Grassi's often-quoted statement that he found amebas in the stools of patients suffering with colitis, secondary to intestinal tumors, we may say that we have had a case of carci-

noma of the cecum in which amebas were found in the stools, and a diagnosis of double infection was established at necropsy.

We are justified, therefore, in concluding with Lafleur and others that many if not all of these cases were based on a faulty diagnosis rather than that the amebas were present in these diseases as harmless commensals. The absence of post-mortem observations is particularly noticeable. To draw the conclusion that the amebas present in the diseases reported were not playing a pathogenic rôle without observation after death must be accepted by those fully acquainted with the nature of the disease as almost entirely valueless. Even were such observations made, the conclusions would be but slightly more justifiable, unless it were shown that the amebas had been propagating in the bowel during life, for, as has already been said in discussing amebas in healthy persons, it is only the *persistence* and not the accidental passage of these parasites through the colon that can have any weight in the consideration of this subject.

Amebas secondary in action.—The opinion that amebas are not primarily pathogenic but may possess the power to alter or intensify the lesions already present in the intestine, is one which was formulated many years ago and which has had not a few ardent supporters; and many able men are still of this opinion.

This idea lends itself readily to the conclusions which may be drawn from many anatomic pictures which, without attributing to amebas some pathogenic action, can not be explained by those who have attempted to prove the innocence of the parasites.

One of the arguments most commonly employed to support this position is the statement that amebas may be found in the ulcers and lesions caused by other well-known etiologic agents. A number of writers call particular attention to their presence in tuberculous ulcers of the intestine, and we can confirm this observation at least to the extent that tubercle bacilli and amebas may be present at the same time in intestinal ulcers.

We have recently performed a necropsy on such a case. There was advanced tuberculosis of the mesenteric glands and of the intestine. The intestinal ulcers in their gross appearance more closely resembled those of intestinal amebiasis, but both microscopic and histologic study of the sections showed the results to be those of a double infection. And why not? From the nature of two such diseases, and from what is coming to light as we learn

more of the nature of amebic infections, such cases should not be any more surprising than are other more or less frequent associations of infectious diseases.

Such double infections by amebas and other factors, particularly those which may produce ulceration in the colon, as typhoid fever, Bright's disease, etc., are not of infrequent occurrence; but this fact can no more be justly used as an argument to prove the secondary actions of amebas than can typhoid ulceration be said to be secondary to malaria, when these two diseases happen to exist together in the same patient.

A less tangible argument which is often used in support of this proposition, is based upon the supposition that amebas are frequently found in the normal intestine, where they do no harm until a "cold," "indigestion," "diarrhea," or what not causes changes in the colon, which enable the amebas to assume a pathogenic rôle. There is evidence that in some cases this is true, to the extent that such conditions may facilitate the propagation of parasites which happen to reach the bowel during such a time; but certainly there is little to indicate that it is more universally so with amebas than in the case of very many of our accepted etiologic agents.

Pathogenic and nonpathogenic amebas.—Since the appearance of the work of Quincke and Roos, Kruse and Pasquale, and others, who furnished evidence in support of their conclusion which appeared satisfactory at that time and which had a tendency to harmonize the two former extremes, many supporters of these ideas have attempted to discover simple means which could be used for diagnostic purposes, in order to distinguish pathogenic from nonpathogenic amebas. This has always been recognized as difficult; but, as it is usually applied at the present time, consists in recognition of certain supposed morphological differences between the parasites and in the unlike results obtained in animal experiments. In general, the larger amebas, some of which contain red blood cells, etc., have been considered pathogenic and to distinguish them they have usually been termed *Ameba dysenteria*. The smaller species, which are considered nonpathogenic, do not contain red blood cells and show other morphologic variations in structure, are quite generally designated *Ameba coli*.

Formerly, writers largely concurred in this opinion, but more extended experience has forced some changes in their views, if not with reference to the general question of pathogenicity and

nonpathogenicity, certainly as to the value of all methods which have heretofore been applicable in distinguishing such differences. Long experience in the examination of stools and in post-mortem examinations have fully convinced us that the size and other morphologic appearances of the amebas have but little, if any, relation whatever to their pathogenesis.

Some of the most persistent and even fatal cases of dysentery may show amebas of not over 10 to 20 μ in diameter in stools during life and in the intestinal ulcers, post-mortem. On the other hand, only very large amebas may appear in some of the cases most amenable to treatment and in fatal cases which show the smallest amount of ulceration. Cultivation of the amebas in artificial media has demonstrated that the variation in size may be influenced at will by a number of conditions.

The value of the inclusion of red blood cells by amebas as a point in differential diagnosis is also exceedingly doubtful. There are many cases of amebiasis which run the regular course and at necropsy show the typical lesions, but in which inclusions of red blood cells by the amebas are not observed in repeated stool examinations during life. There is even evidence to show that the presence of such cells is not an indication of parasitic activity, but rather one of decay or degeneration. As has already been said, the young, active amebas in artificial media are quite select in their diet and among other substances avoid red blood cells, but as degeneration, resulting from toxic effects or other causes, sets in, less selectiveness in the choice of food is manifested and consequently whatever is within reach is taken up by the protoplasm.

The argument purporting to prove the existence of at least two varieties of amebas, based on the presence of harmless ones in the normal intestine and in those of persons suffering from other diseases, has already been discussed.

Another method which has been employed in facilitating a classification of these parasites consists in the results of animal experiments, which will be considered presently. When one regards the contradictory character of the results of experiments made with stools known to be dysenteric, it seems that we may justly conclude that to adduce further data of this nature as a basis for a classification of amebas is unwarranted.

This is true of the whole list of arguments heretofore given which have been used for purposes of differential diagnosis. Neither

one nor all taken together are sufficient to justify the conclusion that there are two kinds of amebas found in the human intestine. *We do not wish to be understood as denying that there are pathogenic and nonpathogenic varieties of amebas; what we desire to say is that, thus far, the existence of such varieties has not been proved, nor are we as yet able to settle the question.* Still our work has been convincing along certain lines. Amebas cultivated from various sources, including the dysenteric intestine, the Manila water supply, lettuce, etc., have proved pathogenic under certain conditions, which reverses the view held of some of those formerly considered harmless.

All amebas are or may become pathogenic. This proposition, pending a complete solution of the problem, is the only safe one to adopt from the standpoint of public health in the Tropics. To admit such a proposition adds much to be explained by natural immunity and other conditions. This is especially true in a country where these parasites are found everywhere—in water, in earth, on vegetables, fruits, the skin, etc., and where, at most, an inconsiderable part of the population use any care whatever to avoid ingesting them, and it therefore must be true that they are taken daily into the gastro-intestinal tract by large numbers of persons. This being so, if all amebas are pathogenic, why have we not more amebic dysentery? And if all are not pathogenic, why do we not find them oftener in the normal intestine?

We have never followed a case which indicated that nonpathogenic amebas were propagating in the intestine, and we are sure that such an occurrence is very rare in the Philippine Islands, where, as has been said, the larger part of the inhabitants take in thousands of amebas daily. For we have had no difficulty in cultivating amebas from the very water they drink, and in producing dysentery in monkeys by the ingestion of cultures from this water under favorable circumstances. If, then, for example, this water contains both harmless and pathogenic amebas, the number of nonpathogenic ones, capable of resisting the stomach acids and of multiplying permanently in the intestine, are very few in comparison with the number of pathogenic ones, capable of resisting the same influences and found associated with the lesions of a pathologic entity.

We are not prepared to state that all cultures of amebas from water would give the same positive results as the one just men-

tioned, but the question is now open to solution by the methods described in this paper. It is perfectly compatible with all the facts known about the infection to consider all amebas pathogenic, although the evidence now at hand does not warrant such a conclusion. The principal points in this connection have already been considered in the chapter devoted to the cultivation of amebas, and need not be repeated here.

Animal experiments.—Animal experiments with substances containing amebas, since Lösch's first case in 1875, have given conflicting results, owing to the necessarily crude methods of inoculation due to the absence of cultures, and these results have been used as evidence both for and against the pathogenic action of amebas. Before commencing a discussion of our work, it seems advisable to note briefly some of the results of others:

Lösch (1875) injected the stools of dysenteric patients into the rectum of four dogs, one of which had previously received an injection of croton oil. One of the dogs which had not received the croton oil developed symptoms of colitis.

Hlava (Uplavici) (1887) introduced dysenteric stools into the rectum of seventeen dogs, obtaining positive results in two cases. Six cats were similarly treated, with four positive results. Eight rabbits and six guinea pigs gave no symptoms of infection.

Kartulis (1889) injected stools containing amebas into the rectum of dogs, monkeys, guinea pigs, and rabbits, with negative results. Cahen (1891) had similar results with cats.

Calandrucio (1890) swallowed encysted amebas and at the end of twelve days found similar forms in his normal stools.

Kovacs (1892) introduced amebas into the cecum and rectum of dogs without obtaining any effects attributable to these parasites.

Quinke and Roos (1893) injected amebic dysenteric stools into eight cats, six of which died in from two to three weeks with amebic dysentery. Typical ulcerations containing motile amebas were found at necropsy.

Kruse and Pasquale (1893) produced dysentery in eight out of sixteen cats by rectal injections. Similar results were obtained with bacteria-free pus from liver abscesses.

Zancoral (1893) says that it is difficult to produce dysentery in animals by means of rectal injections. He succeeded, however, in giving rise to dysenteric symptoms in this manner—a result which he was also able to produce by the use of liver abscess pus free from amebas.

Roos (1894) injected dysenteric stools into the rectum of eight cats while they were under ether anesthesia. Six of these contracted fatal cases of dysentery, dying in from ten to twenty-two days with the presence of amebas in the intestinal contents.

Gasser (1895) produced dysenteric lesions in cats by means of rectal injections.

Fijardo (1896) injected the contents of liver abscess and amebic stools into the rectum of cats. In no case was a typical infection produced, but one of the animals which had been treated with the feces died in four days without dysenteric symptoms or lesions at necropsy, and the other in twenty-four hours after passing bloody mucus stools containing amebas. At the post-mortem examination of the latter case no ulceration was observed, but the mucosa of the rectum was swollen and red.

Strong and Musgrave (1900) obtained positive results in several cats by the rectal injection of stools from dysenteric patients, and in one by the injection of culturally bacteria-free liver abscess pus. The post-mortem examination in these cases showed typical amebic ulceration.

Jäger (1901) made injections of similar stools into four cats, three of which were infected thereby, but the fourth of which showed no symptoms of the disease. One of the animals which died at the end of a month revealed typical amebic ulceration at necropsy.

Ucke (1902) infected two young cats by rectal injections of dysenteric stools. Both of the animals died of inanition. At necropsy neither ulcerations nor amebas were found in the intestinal canal, although the organisms were present in the stools during life.

Huber (1903) infected dogs by feeding them stools containing encysted amebas.

Harris (1901) produced dysentery and liver abscesses in puppies by rectal injections of feces containing amebas, but when mixed culture of all the bacteria which grew on plates from similar stools were used in a similar manner, no symptoms were produced. He therefore concluded that amebas were the etiotic factors in this disease.

Cunningham, Grassi, Calandrucio, and others maintain that the great resistance of amebas in the encysted stage may play a significant part in the transmission of infection from one animal to another. This is borne out by the experiments of Quincke and Roos (1893), who fed two cats with stools containing amebas, which had been allowed to encyst by leaving them at room temperature for from two to nine days after being passed. Both of these animals developed dysentery, showing the presence of amebas in the stools and at necropsy amebic ulceration of the colon. In similar experiments with motile amebas no infection resulted.

As to the other side of the question, there have been a large number who have failed to obtain positive results on repeating the experiments here enumerated. At any rate, at present, as Janowski expresses it, there is nothing more to be gained by this sort of work.

It seems that those who previously believed in the pathogenic rôle of amebas have already become convinced of the reliability of many of these experiments, while those who do not accept the etiologic rôle of these parasites will never give up so patent an argument as is offered by the necessary crudeness of all experiments made with stools or liver abscess contents.

It seems to us that the sum total of this work, particularly that part in which positive results were obtained by the injection of

amebic pus culturally free from bacteria, is all but convincing, but in order to furnish sufficient proof of this fact to the unsatisfied, it will be necessary to adopt other methods of experimentation, and the natural method is that of culture. Such experiments have apparently heretofore not been made with any great frequency.

Kartulis (1891) asserted that he had produced dysentery in cats by rectal injections of pure cultures of amebas isolated from a liver abscess as well as with impure cultures of amebas obtained from the dysenteric intestine and grown upon his straw-infusion medium. Feeding similar cultures gave no results. Kartulis also produced temporary diarrhea in two dogs by rectal injections of amebas grown together with bacteria in sterilized rabbit dung.

Vivaldi (1893) injected cultures of amebas, which he had grown in straw infusion, into the rectia of cats, and diarrhea followed. At necropsy catarrh of the colon was observed, but there was no evidence of ulceration.

Casagrandi and Barbagallo (1897) conclude that amebas which have been grown on artificial media are not parasitic, because such cultures do not produce the disease in cats.

Zaubitzer failed to obtain pathogenic action with cultures of his straw-infusion amebas. After feeding guinea pigs with these cultures, he was unable to find the parasites in the stools. But when the experiments were repeated in frogs, encysted amebas were found in the stools and recovered by culture. He thoroughly lavaged the bowels of cats and then introduced cultures and stitched the anus, but obtained negative results.

Fiori fed cultures of the *ameba coli* of Celli and Fiocca, isolated from the water of Alexandria, to a patient suffering with chronic diarrhea, which at intervals was dysentery. Amebas were found in the stools for a few days, but the course of the disease was not altered.

Kartulis finally injected straw-infusion cultures of what he considered to be dysenteric amebas into the rectum of a cat and closed the anus by suture. On the sixth day there were mucus stools. The animal died of dysentery, but no necropsy was performed.

Our experiments with cultures of amebas on animals, a number of which are briefly described below, have been quite convincing as to the etiologic rôle of some amebas. These results have been particularly satisfactory with monkeys, but nothing definite has been gained by working with cats, dogs, and other animals. Even with monkeys, infections have not been constant, and the lesions produced in them, while satisfactory, have not been so extensive as those usually seen at necropsy in man. Otherwise the specimens obtained have had the macroscopic appearance of amebic infection and the parasites have always appeared in the contents of their intestines. A study of sections from the colon has confirmed the nature of the infection in each instance.

It will be noticed in looking over the brief protocols that most of our animals were killed rather than allowed to die. This was done in order to secure fresh necropsies when it appeared that an animal would not live until the following day. Just why the experimental disease when once established, should run so rapid a course in monkeys, we do not know; but it corresponds in every particular with the cases of natural infection which have come under our observation in the same class of animals.

During a recent epidemic of pneumonia among the inmates of Bilibid Prison we were able to secure an abundance of material particularly showing the very early lesions of amebiasis; and these lesions so closely resemble many of those seen in our experimental animals that it makes us all the more certain of our results in the latter. We do not wish at this time to go into the pathology of this infection, much of which remains to be described. The classical lesions of advanced cases are well recorded, and some notes on earlier ones have been carefully made by Councilman and Lafleur, Harris, Diamond, and others; *but there is undoubtedly a somewhat definite preulcerative pathology and varying stages and varieties of early ulceration, as well as a greater variety of the more advanced lesions than has generally been recognized.* The materials which we now have would indicate that both the macroscopic and the microscopic pictures show variations as great as those observed in the intestinal lesions of typhoid fever, including the nonulcerative type.

It will also be noticed in looking over the attached protocols, that the incubation period in monkeys is a rather long one, and in those animals which gave satisfactory results from a single feeding it is remarkably constant. This is borne out by one human infection and offers still further evidence of the latency often seen in the naturally contracted cases. The symptoms in experimental cases in monkeys closely resemble those observed in man. Anemia and emaciation are constant, and are manifested before the development of diarrhea. In some of these the onset is rather sudden with severe diarrhea, which continues almost unabated until death, but as in the case of man, it more often starts as an intermittent diarrhea, which becomes more severe with each exacerbation.

In most of our experiments the animals have been under observation during from ten to thirty days. A large number of monkeys,

including those not inoculated and the ones used for other experiments, have been constantly kept in the same room with our animals under identical surroundings, and of more than one hundred of these, and of probably two hundred and fifty which have been passed through the animal yards at the Serum Laboratory, only three have been found attacked with spontaneous amebic infection, two of these developed in animals kept in cages beside those in which our experimental animals were confined.

We have occasionally observed cases of naturally contracted amebiasis in monkeys at other times, but such instances are not frequent, and so far as we have been able to trace, occur only in animals which for some time have been in captivity and have thus had opportunities similar to those of human beings to contract the infection from sources which to them are unnatural. At any rate, considering the care which we have taken to see that our experimental animals were free from infection at the time of feeding, natural infection could hardly be entered as criticism of our work. Neither could it be said that monkeys are natural hosts of amebas, for these parasites are not often encountered in their stools, and the lesions found in the naturally contracted cases are in every particular like those of the experimental ones.

There is one point in these experiments which indicates that a lowered vitality may have to do with the certainty and the intensity of the infection in the individuals exposed to it naturally. When animals which have been fed amebas are injected intra-abdominally with cultures of bacteria which of themselves would cause illness without death, the incubation period of the amebic infection has been considerably shortened.

Some of our monkeys which have been fed amebas, and which have developed diarrhea with the presence of the parasites in their stools, have recovered. This is, no doubt, occasionally true in the naturally contracted disease in man.¹

¹ Owing to the extremely chronic character of many of these infections, characterized as they are by long periods of quiescence between exacerbations, it is difficult to find the earliest moment when recovery is complete. We have, however, been convinced that such is occasionally the case even in the *unacclimated* Caucasian, and more often so in the native, because we have observed such recoveries in one Caucasian and three natives, in all of which the diagnosis was unquestionable, treatment was never instituted, and more than a year has elapsed since there was clinical evidence of the disease, or amebas in the stools.

We have not succeeded in infecting a monkey with young cultures of motile amebas, though in some of the experiments the ones used were but a few days old and cyst formation hardly complete.

Some interesting facts have come to light regarding the rôle of the symbiotic bacteria which are present in the mixed cultures used in our experiments. Attempts to reclaim these bacteria from stools of the infected animals have not been made in all cases, but in some instances such bacteria fed have not been isolated, indicating that the symbiosis had been changed in the intestine of the animal. This was not found to be true, however, in the case of man. The yellow, pigment-producing bacillus in symbiosis with the amebas in the culture ingested by the subject was recovered from the stool after symptoms of dysentery had developed.

*Monkey No. 536*¹ had been under observation in the laboratory for fifteen days, during which time diarrhea was not present and amebas were not found in the stool by microscopic examination.

On February 9, 1904, it was given by stomach tube 10 cubic centimeters of a distilled water emulsion of old encysted cultures of *Ameba* 11524 in symbiosis with *Spr. cholerae*. No further treatment of any kind was administered, and the animal apparently remained well until the twenty-seventh day after feeding, when a slight diarrhea was noted. By the thirty-third day this diarrhea had become very decided; there was considerable mucus, a few red blood cells, and fair numbers of amebas in the stools. Stained preparations and peptone cultures gave no evidence of the presence of *Spr. cholerae*. From the thirty-third to the thirty-seventh days the diarrhea abated somewhat, but by the fortieth day had become a moderate clinical dysentery, which progressed until the forty-seventh, when the animal was killed.

At necropsy, which was performed immediately, emaciation and anemia were marked. The walls of the colon were thickened, the mesocolic glands slightly enlarged, the subserous vessels injected, and the omentum adherent to the cecum. The colon contained fluid material with mucus and blood, and showed a hemorrhagic catarrh of the mucosa throughout. Ulcers from 2 to 5 millimeters in diameter were found scattered throughout the mucous membrane of the colon. These ulcers were rather superficial in character and contained numerous amebas, some of which enclosed from one to three red blood cells. Cholera spirilla were not found. The amebas microscopically, resembled those which were fed and were not reclaimed by culture. (See fig. 3, Pl. I.)

A monkey similar to the one above mentioned was fed repeatedly large doses of *Spr. cholerae* of the same stem as that used in the preceding culture by Dr. W. B. Wherry, but without the production of diarrhea.

¹ The monkeys used in these experiments were of the two varieties native to the Island of Luzon, *Macacus cynamolgus* and *Macacus philippinensis*.

Monkey No. 442, on October 20, 1903, was fed 10 cubic centimeters of an emulsion of an old encysted culture of *Ameba* 11524. On October 22 this feeding was repeated. The animal remained well until November 21, when it was given in the abdominal cavity 5 cubic centimeters of an emulsion similar to that above mentioned. On December 2 it was injected with 10 cubic centimeters in the abdominal cavity, and on December 21, 20 cubic centimeters. In the latter part of December there was diarrhea for a few days. On January 6 diarrhea again developed, the stools containing mucus, a few red blood cells, and fair numbers of amebas. By January 22 the animal had become very much emaciated and anemic, passing frequent bloody stools, which contained large numbers of amebas.

On this date chloroform was administered and the necropsy performed immediately. There was marked emaciation and anemia. The inguinal and mesenteric glands were slightly enlarged. Old chronic peritonitis was evident, the omentum being firmly adherent to the intestines, the surfaces of which also adhered to one another in many places. The large intestine showed hemorrhagic catarrh throughout. Motile amebas, some of them containing red blood cells (one of them contained as many as eight), were found in the rectum and the cecum. The walls of the colon were thickened. The solitary follicles were enlarged and capped in places with superficial necroses. There were a few small superficial ulcers, measuring from 2 to 5 millimeters in diameter, in the rectum and the cecum. The anatomic picture in general did not differ from that seen in man when death occurs during the early stages of the disease. Amebas were not obtained in cultures from the colon.

Monkey No. 464, on November 18, was given subcutaneously 4 cubic centimeters of an emulsion of an old encysted culture of *Ameba* 11524; on November 24, 10 cubic centimeters of a similar emulsion; and on December 7, 20 cubic centimeters. On December 9 the abscesses which had formed at the points of the injections were opened and found to contain numerous amebas, which grew on culture. On December 12 the serum from this animal showed no parasitocidal action on the amebas cultivated from the abscess. On December 21 another subcutaneous injection of 15 cubic centimeters was given. On the 24th diarrhea developed. From January 1 to 7 the dejecta were dysenteric in character and quite numerous.

On January 12 the animal was chloroformed and necropsy performed at once. Emaciation and anemia were marked. The large intestine contained a considerable amount of mucus and a small amount of blood, and on microscopic examination showed large motile amebas. The walls of this bowel were thickened, and the mucous membrane showed hemorrhagic catarrh. The solitary follicles were slightly swollen, and there were a few small superficial ulcers. Amebas were not obtained by culture from the colon. We believe this animal infected itself from the abscess by scratching and licking the wounds and the fingers.

Monkey No. 678 was a healthy animal which had been under observation for ten days, during which no amebas were found in the stools. On May 2, 6, and 9 increasing doses of *Spr. cholerae* were injected subcutaneously. On May 11 a "pure mixed culture" of *ameba* 11524 and *Spr. cholerae* was

injected into the right lobe of the liver. On May 15 the animal was chloroformed and necropsy performed immediately.

The liver appeared normal, except for slight hyperemia at the point where the inoculation needle entered. Neither amebas nor cholera spirilla were found in this organ. The mucosa of the colon was observed to be congested, and there were small hemorrhages into some of the solitary follicles. A few of the latter showed superficial necroses with the presence of fair numbers of amebas.

Monkey No. 371, on September 16, was given, by high enema, 5 cubic centimeters of an emulsion of an old encysted culture of *Ameba* 11147. One month later the animal developed a slight diarrhea, and at necropsy, performed immediately after death by chloroform, a few amebas were found in the contents of the colon. There was considerable mucus in the cecum and the ascending colon, but no blood and no macroscopic lesions were evident.

Monkey No. 493, on December 12, was given in the abdominal cavity 5 cubic centimeters of an old encysted culture of *Ameba* 11147. Another injection of 15 cubic centimeters was made on December 21. On January 6 the animal was killed, and no lesions were found at necropsy.

Monkey No. 555, on February 23, was fed 5 cubic centimeters of an emulsion of an old encysted culture of *Ameba* 11147 together with *Spr. cholera*. On March 14 diarrhea developed, and on the 17th the animal died with clinical symptoms of dysentery.

At necropsy there was marked emaciation and anemia. There was catarrh of the colon throughout, this being most marked in the cecum and the ascending colon, where there were a very few small ulcers. The liver contained several small abscesses, measuring from 1 to 3 millimeters in diameter, which enclosed bodies resembling amebas, but which were not motile. The animal had been dead eight hours at the time of necropsy.

Monkey No. 479, on November 30, was given subcutaneously 2 cubic centimeters of an emulsion of *Ameba* 11147. On December 2 the animal died of dyspnea. At necropsy there was a small amount of dirty viscid pus at the point of inoculation, which, however, contained no amebas microscopically. There was an abscess about 2 millimeters in diameter in the left lung, which contained numerous motile amebas. The colon was normal and no other significant changes were found in the body. Amebas were not obtained by culture from the contents of the lung abscesses.

Monkey No. 553 had been under observation for one month, and during this time had shown no diarrhea, and its stools contained no amebas. On February 23, it was fed 10 cubic centimeters of an old culture of *Ameba* 25624. The animal died on April 5, after a diarrhea of two weeks' duration. Necropsy was performed ten hours post-mortem. Emaciation and anemia were very marked. The liver and heart showed fatty degeneration. There was slight general lymphatic enlargement. The colon contained fluid material, with considerable mucus and some blood. The mucosa throughout the colon was very much swollen and in a few places showed superficial necroses. Amebas similar to the ones which had been fed were present in the stools during life, but on account of delay in necropsy the contents of the colon were not examined post-mortem.

Monkey No. 554 had been under observation for twelve days, and during this time had shown no diarrhea and stools contained no amebas. On February 22 it was fed 10 cubic centimeters of an old encysted culture of *Ameba* "water." About one month later diarrhea developed, and the animal became very much emaciated. On March 26 it was killed by chloroform. At necropsy, which was performed immediately, the walls of the colon were found to be thickened. There were several small ulcers in the cecum as well as a sloughing of the mucous membrane over a considerable area near the splenic flexure. Numerous amebas, some of them enclosing red blood cells, were found in the contents of the bowel. (See fig. 2, Pl. I)

Monkey No. 526, on January 7, was chloroformed and the cecum exposed, after which 10 cubic centimeters of an old culture of *Ameba* "water" was injected from a hypodermic syringe into this organ. On February 4 a violent diarrhea developed, and the stools contained numerous amebas, some of which enclosed red blood cells. The animal was killed by chloroform and necropsy performed immediately. The mesocolon was slightly enlarged and pale. The mesenteric and intestinal blood vessels were very much injected, and there were adhesions around the operation wound (which healed by first intention). The colon contained semifluid feces with some mucus and blood. The cecum splenic flexures showed marked hemorrhagic catarrh. The solitary follicles were swollen throughout, some of them showing early necrosis, and others, beginning small ulcers 1 to 3 centimeters in diameter. These little ulcers contained large numbers of amebas, which were also found throughout the intestinal contents. Amebas grew out in culture from the ulcers of the colon.

Monkey No. 538, on February 10, was given in the abdominal cavity 10 cubic centimeters of an old culture of *Ameba* "water," which had been grown from monkey No. 526. By February 25, a large abscess had developed at the point of inoculation, continuing to increase in size until March 8, when the animal was killed. The abscess contained thick, yellowish pus, and bodies resembling encysted amebas; but there were no motile parasites. The colon was normal and free from amebas.

Monkey No. 537, on February 10, was inoculated in the liver with 1 cubic centimeter of a suspension of an old culture of *Ameba* "water" obtained by culture from the colon of monkey No. 526. On February 16 the animal was killed and necropsy performed immediately. The under surface of the diaphragm was much congested and adherent to the dome of the liver over an abscess about 1 centimeter in diameter. This abscess was quite superficially situated and had the gross appearance of an amebic abscess of the human liver. Microscopically the pus was found to contain bacteria and amebas, some of which enclosed red blood cells. The colon was normal and free from amebas. Amebas were not grown by culture from the liver abscess.

Monkey No. 542, on February 16, was inoculated into the stomach with cultures from the liver abscess of monkey No. 537. This abscess had been produced by cultures of amebas from the stools of monkey No. 526, which had been in turn infected by feeding with old cultures of *Ameba* "water." On February 23, the animal was killed. There were numerous actively

motile amebas in the cecum, but no macroscopic lesions were found in the colon, and no attempt was made to reclaim these amebas by culture.

Monkey No. 518, after being proved free from infection, on December 29, 1903, was inoculated subcutaneously with 10 cubic centimeters of an old culture of *Ameba* "water." On January 2 an abscess formed, and on January 22, diarrhea developed with large motile amebas in the stools and continued until January 27, when the animal was killed. Lesions of an early amebic infection were present.

One of the ulcers 6 millimeters in diameter, was situated on the ileocecal valve, and another one, a little smaller, in the cecum. Amebas were present in the ulcers and were obtained by culture. As with the two other monkeys already mentioned, it is believed that this animal infected itself from the abscess, a rational explanation to those familiar with the action of these animals under such conditions.

Monkey No. 517, on December 29, was inoculated in the abdominal cavity with 15 centimeters of *Ameba* "water." On January 3 the abscess which had formed at the point of inoculation ruptured, and its contents were found to contain amebas. The animal was killed and the intestinal canal was found to be free from amebas and normal in appearance.

Monkey No. 534.—On March 24, 2 cubic centimeters of an old culture of *Ameba* "lettuce" was injected into the liver along with a bacillus from the city water supply. On April 10 the animal was killed. At necropsy the intestine was found to be normal. There were two liver abscesses, the larger one being 10 millimeters in diameter. On section these abscesses had the gross appearance of an amebic abscess of the human liver. Amebas and bacteria were obtained by culture from the contents of one of the abscesses.

Monkey No. 552, after being proved free from clinical symptoms and amebas, was inoculated on February 23, in the stomach with the aid of a catheter, with 6 cubic centimeters of an old culture of *Ameba* "lettuce." On March 14, diarrhea developed, but no amebas were found. On March 17, the stools contained considerable mucus and blood, and amebas were present in large numbers. On March 19 the animal was killed. Necropsy showed hemorrhagic catarrh of the entire colon, which contained a large amount of bloody mucus in which were found numerous amebas. There was a general thickening of the walls of the colon, and numerous small superficial ulcers were present. (See fig. 1, Pl. I.)

Monkey No. 531, on January 27, was inoculated subcutaneously with 1 cubic centimeter of a stool containing large numbers of amebas. An abscess developed. The animal was killed on February 9. The intestine was found to be normal. The subcutaneous abscess contained amebas, which were reclaimed by culture.

Monkey No. 357 died from spontaneous diarrhea on August 30, 1903. At necropsy there was found to be catarrh of the colon, but no ulceration. No amebas were obtained by culture.

Monkey No. 558, which had been kept in a cage adjoining experimental animals, spontaneously developed profuse diarrhea on March 25. The stools contained numerous amebas. Death occurred from dysentery on March 26. At necropsy there was found tuberculosis of the lungs and

and spleen. The colon showed hemorrhagic catarrh, and contained numerous small ulcers, which enclosed amebas.

Dog No. 441, on October 20, was fed old encysted cultures of Ameba 11524. This treatment was repeated on October 22 and 26 and November 9 and 11. There was no diarrhea, and no amebas were found in the stools up to this time. On November 12 the dog was given in the abdominal cavity, 10 cubic centimeters of an emulsion of a similar ameba in melted agar. The animal died on November 24, of acute general peritonitis. No amebas and no lesions were present in the colon.

Dog No. 553, on January 29, was given by stomach tube 10 cubic centimeters of an emulsion of an old encysted culture of Ameba 11524. There was no diarrhea, but anorrhexia, anemia, and emaciation developed early and were progressive until the animal died on March 14. At necropsy, which was performed ten hours post-mortem, there was marked catarrh of the entire gastro-intestinal canal, but there was no ulceration, and no amebas were found in the contents of the colon.

Cat No. 366, on September 4, was fed 5 cubic centimeters of an old encysted culture of Ameba 11147. This treatment was repeated on September 7, 9, and 15. The animal remained well until October 19. On this date and on November 24 additional treatments were administered; but there occurred no symptoms of bowel trouble up to December 4, when the animal was killed. At necropsy no amebas were found in the intestinal contents, and no macroscopic lesions were present.

Cat No. 363, on September 1, was fed 5 cubic centimeters of a twenty-four-hour bouillon culture of Bacillus 9650b. This treatment was repeated on the second and third. On the fourth 5 cubic centimeters of an emulsion of an old culture of Ameba 11147 and of Bacillus 9650b was introduced into the stomach by tube. This treatment was repeated on September 7, 9, and 15. On October 15 diarrhea developed, and on the nineteenth the stools contained considerable mucus and some blood. The animal was chloroformed on the twentieth and necropsy performed immediately. Aside from moderate anemia and emaciation, there were no lesions of importance except in the colon, which contained a considerable quantity of mucus, motile amebas, and some blood, as well as numerous small punctate hemorrhages. There was some swelling of the solitary follicles with superficial necrosis, but no distinct ulceration.

Cat No. 350, on October 20, was given by high enema 5 cubic centimeters of a young growing culture of Ameba "water." This treatment was repeated on October 25, 28, and 31 and November 2. No diarrhea or other symptoms were produced, but the animal was killed on November 4, and necropsy was performed at once. The lower portion of the colon appeared normal, but the cecum contained a considerable amount of semiliquid fecal matter and mucus. There were no macroscopic lesions of the mucosa, and no amebas were found.

Cat No. 353 was given 5 cubic centimeters of an old culture of Ameba "water" by high rectal tube. This treatment was repeated on alternate days until five injections had been given. During the next month there were slight attacks of diarrhea with some emaciation; but no amebas were

found in the stools. At necropsy the cecum contained some mucus and some blood, but no parasites were found. The walls of the colon were thickened, without other decided evidences of infection.

Cat No. 349.—The large bowel of this animal was cleaned with glycerin enemas, followed by lavage with warm water. After one hour 5 cubic centimeters of an old culture of *Ameba* "water" was introduced by means of a catheter high up into the colon, and a similar injection was thereafter given every four days for two weeks. No symptoms of dysentery were produced. At autopsy, made two months after the first injection, no lesions and no amebas were found in the colon.

A healthy man, kept under observation for ten days, during which time his stools were not found to contain amebas either microscopically or culturally, on repeated examinations following cathartic doses of Rochelle salts, ingested three ordinary gelatin capsules, which contained scrapings from three-week-old cultures of *Ameba* 11524 in symbiosis with a bacterium which had been proved harmless for animals and man.

On the twelfth day after treatment there was slight diarrhea, and amebas was the first time present on microscopic examination. From this time until the twentieth day there was mild intermittent diarrhea. On this day some tenesmus was complained of and the stool contained considerable mucus and a small amount of blood in addition to large numbers of amebas, some of which enclosed red blood cells. This evidence was considered sufficient to establish the diagnosis, and the patient was placed under treatment. Both the ameba and the bacillus introduced were reclaimed by culture on the day after the development of diarrhea.

IV. CONCLUDING REMARKS.

The term "amebiasis," which has been introduced in this article, denoting an infection with amebas, is comparable in its application to filariasis, trypanosomiasis, uncinariasis, etc. It is not open to the objections so frequently offered to "amebic dysentery," "amebic enteritis," and the other names usually given to the disease.

Amebas, when present in water, soil, and other places outside the animal body, may almost constantly be secured in culture, and by the methods described in this paper pure species in pure cultures of bacteria may be obtained. Such pure species of amebas may be secured by comparatively simple means, and their isolation with pure cultures of bacteria—the "pure mixed culture" of Frosch—by an equally practical technique.

Amebas may be cultivated from dysenteric stools and from ulcers in the human bowel. This has been done in 2 per cent of the cases by the direct inoculation of the material containing the amebas on the surface of the media recommended. Positive results are more frequently obtained (30 per cent) when the media are

simultaneously treated with certain bacteria. They may be grown in a still larger per cent of cases (60) when the media are smeared with the bacteria isolated from the same source as the amebas.

Living bacteria or other microorganisms seem to be necessary to the existence of the amebas under artificial condition.

Amebas show a certain selectiveness for symbiotic bacteria, and this selectiveness may be increased and changed on artificial media.

The passage of cultivated amebas through the animal organism, either subcutaneously or by the gastro-intestinal canal, increases the selectiveness of the parasite for symbiotic organisms and the difficulty of its growth on artificial media. This phenomenon appears to be progressive with successive animal inoculations, until in some cases the cultivation becomes impossible. If these injections are made into the liver of monkeys, the progressive difficulty of growth increases rapidly with subsequent animals, and the cultivation on artificial media soon becomes impossible with the means at command.

There is as yet no way of determining beforehand what bacteria will furnish satisfactory symbiosis; but the variety of those which may act satisfactorily with amebas in the human intestine is probably large and includes many of the ordinary and well-known organisms, both pathogenic and nonpathogenic. The same one, however, is not satisfactory for all amebas.

Amebas have not as yet been grown in pure culture. Whenever they have been freed from other microorganisms by various methods, they have refused to multiply on any known medium.

There are certain differences in the resistance of different cultures of amebas to certain physical and chemical influences.

The cultivation of pure species of amebas has offered strong evidence of the plurality of species of these protozoa, and this plurality apparently extends to those which produce infection in man.

The transient appearance of amebas in the normal intestine is a much rarer occurrence than it is generally believed to be, and their persistence in this location without the production of lesions for a time greater than that of the known latency of some cases of infection has not been shown.

Other evidence brought forward to show the harmlessness of amebas is not conclusive, and certainly in the Tropics the

appearance of amebas in the stools should be sufficiently diagnostic for the institution of therapeutic measures regardless of the nature of the clinical symptoms.

Infection in the colon of monkeys follows the feeding of amebas under certain described conditions, and such pathogenic ones may be cultivated from a number of sources.

In one instance amebas cultivated from the dysenteric intestine with a certain bacillus produced clinical amebiasis by ingestion in man; while the cultures of this bacillus alone failed to produce such symptoms.

Amebas are the etiologic factors in the disease generally known as amebic dysentery, and by following the methods described in this paper, such amebas may be grown on artificial media, and the disease reproduced in monkeys and man by the ingestion of these cultures. Amebas may be reclaimed by culture from the stools or the intestinal ulcers.

In closing we wish to express our gratitude to Dr. Paul C. Freer, Superintendent of Government Laboratories, for his constant advice and aid in editing the manuscript; to Dr. W. R. Moulden, resident physician to Bilibid Prison, for many courtesies, and to the following other members of the staff of the Bureau of Government Laboratories for their assistance; Miss Mary Polk, librarian, for editing the literature; Mr. Chas. Martin, photographer; Mr. C. J. Arnell, translator; and Mr. T. Espinosa, artist.

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[Compiled and edited by Miss Mary Polk, librarian, Bureau of Government Laboratories.]

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EXPLANATION OF ILLUSTRATIONS.

PLATE I. Illustrating the appearance and early lesions of experimental amebiasis in monkeys.¹

Fig. 1. Section from the colon of monkey No. 552 which was fed 6 cubic centimeters of an emulsion of Ameba "lettuce." Diarrhea developed on the nineteenth day and the animal was killed on the sixth day thereafter. Drawing by T. Espinosa.

2. Section from the colon of monkey No. 554 which was fed 10 cubic centimeters of an emulsion of Ameba "water." Diarrhea developed on the thirtieth day and the animal was killed on the fourth day thereafter. Drawing by T. Espinosa.

3. Section from the colon of monkey No. 536 which was fed 10 cubic centimeters of an emulsion of Ameba 11524. Diarrhea developed on the twenty-seventh day and the animal was killed on the twentieth day thereafter. Drawing by Dr. W. B. Wherry.

FIGS. 1, 2. Plate cultures, illustrating the method of securing cultures of amebas with pure cultures of bacteria, Photograph by Martin.

3. Showing the spread of amebas and bacteria on plate cultures. The amebas are seen as white spots in the margins and points of the more homogeneous bacterial growth. The peculiar character of the spread of the combined growth is due to the wanderings of the amebas and the growth of the bacteria carried along by them. See page 29. Photomicrograph by Martin from a twenty-four-hour-old plate culture of Ameba 11524, in symbiosis with *B. coli*. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

4. A forty-eight-hour-old plate culture of amebas, illustrating the distribution of amebas on the plate in the margins of the growth where the bacteria are not numerous. Encysted forms as well as those in all stages of motion are shown. Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

5. An eight-day-old plate culture of amebas, showing the "agglomeration" of amebas sometimes seen in cultures. The amebas are all in the "round stage." Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)

¹For a full description of the experiments see the text on pages 70-76.

- FIG. 6. A three-day-old culture of amebas, showing their rapid multiplication in two colonies of air bacteria which developed in the plates after thirty-six hours. This ameba was afterwards isolated in pure culture with the bacteria shown. Photomicrograph by Martin. (Zeiss objective AA, ocular No. 4. Bellows, 30 centimeters.)
7. A photomicrograph of a cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524 stained by the Wright-Romanowsky method for blood. The contractile vacuole contains a bacterium. Photomicrograph by Martin. (X about 900.)
 8. A cover-glass impression from two twenty-four-hour-old plate culture of Ameba 11524 stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 900.)
 9. A cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524 in symbiosis with B. 12935. Stained by the Wright-Romanowsky method for blood. The nucleus and the so-called spores and vacuoles are distinctly shown and a granular appearance of some of the so-called spores is also well shown. Photomicrograph by Martin. (X 2000.)
 10. A cover-glass impression from a twenty-four-hour-old plate culture of Ameba 11524, stained by the Wright-Romanowsky method for blood. The nucleus, vacuoles (?), spores (?), and and bacteria are shown. Photomicrograph by Martin. (X 2000.)
 11. A sterile-water emulsion of Ameba 11524 from a twelve-hour-old plate. Unstained preparation. Photomicrograph by Martin, instantaneous exposure. (X 800.)
 12. Ameba water. Manner of preparation and amplification of the same as in fig. 11. Photomicrograph by Martin.
 13. A section from a monkey's intestine, showing the distribution of amebas in the preulcerative lesions. Photomicrograph by Martin. (X about 500.)
 - 14, 15, 16. Cover-glass impressions from twenty-four-hour-old plate cultures of Ameba 11147 stained by the Wright-Romanowsky method for blood. Fig. 14 illustrates the rather dense ectoplasm often noticed in unstained specimens of this ameba. Figs. 15 and 16 show forms somewhat suggesting conjugation. Photomicrographs by Martin. (X about 900.)
 17. Cover-glass impression from a forty-eight-hour-old plate culture of Ameba 25624 stained by the Wright-Romanowsky method for blood. The "agglomeration" and distribution of chromatin is well shown. Small masses of chromatin are also shown outside the amebas. Photomicrograph by Martin. (X about 850.)
 18. A cover-glass impression from a thirty-six-hour-old plate culture of ameba lettuce stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 850.)

- FIGS. 19. Cover-glass impression from a twenty-four-hour-old plate culture of ameba lettuce stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 900.)
20. A cover-glass impression from a three-day-old plate culture of Ameba "G" stained by the Wright-Romanowsky method for blood. Photomicrograph by Martin. (X about 800.)
21. Fresh distilled-water preparation from a six-month-old culture of ameba water in symbiosis with a pure bacterial culture. Photomicrograph by Martin. (X about 700.)
22. Fresh distilled-water preparation from a twenty-four-hour-old culture of Ameba 25624 stained *in vivo* by a dilute solution of neutral red, and photographed after all the parasites have assumed the "round stage." Photomicrograph by Martin. (X about 700.)
23. Distilled-water preparation from a six-month-old culture of Ameba 11147, showing the radiations formed by the bacteria along the cyst walls. Photomicrograph by Martin. (X about 650.)
- 24, 25. Distilled-water preparations of Ameba 11524 from young cultures. Stained *in vivo* by dilute solutions of neutral red, and photographed after all the parasites have become round or encysted. Photomicrograph by Martin. (X about 900.)
- 26-32. Illustrating various pictures of encysted forms of Ameba 11524, which, taken consecutively, indicate very strongly one cycle in the life of this parasite.

Fig. 26 illustrates a one-month-old culture containing round forms and both young and mature cysts.

Fig. 27 is from a six-month-old culture (same as fig. 26), showing old cysts.

Fig. 28 is from a three-hour-old transplant from the preceding culture (fig. 27), showing the filling out, nuclear prominence, and other early changes preparatory to further multiplication.

Fig. 29 is also from the preceding culture five hours after transplantation to fresh medium.

Figs. 30, 31, and 32 are from the same transplant at a still later time, and show more advanced formation of the young amebas and a gradual thinning of the cyst wall on the side which, in fig. 32, has reached a point where rupture of the cyst wall is complete.



Fig. 1



Fig. 2

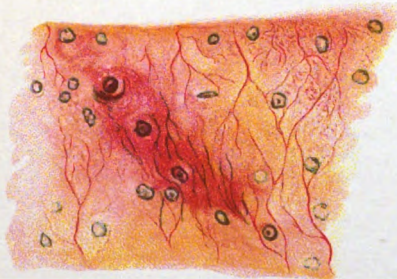


Fig. 3

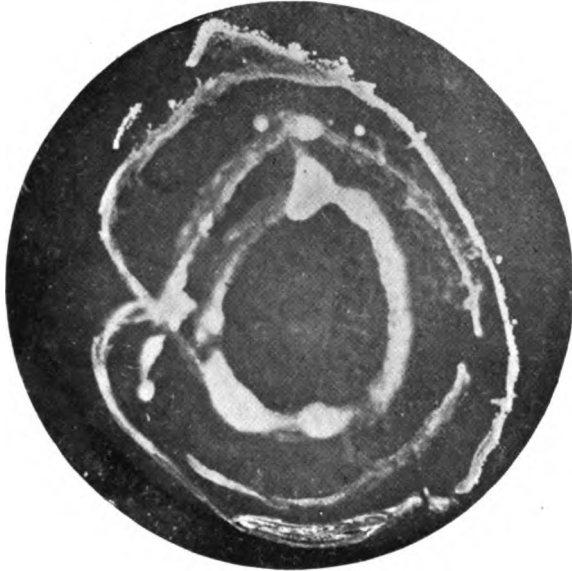


FIG. 1.



FIG. 2.



FIG. 3.

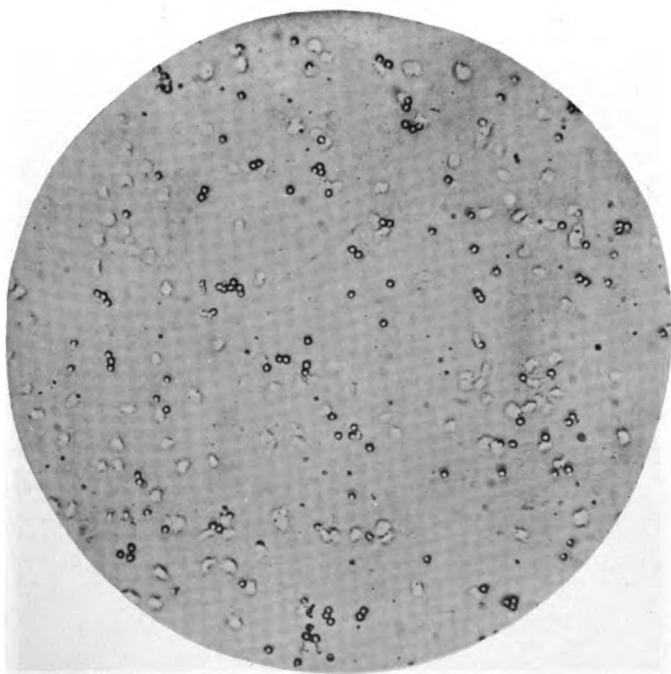


FIG. 4.

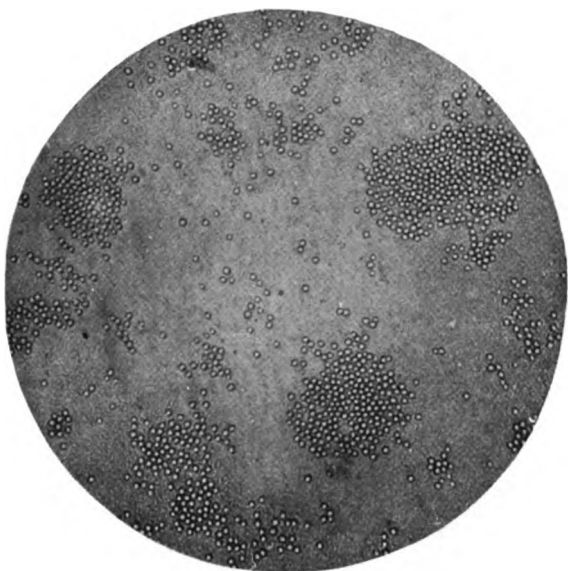


FIG. 5.

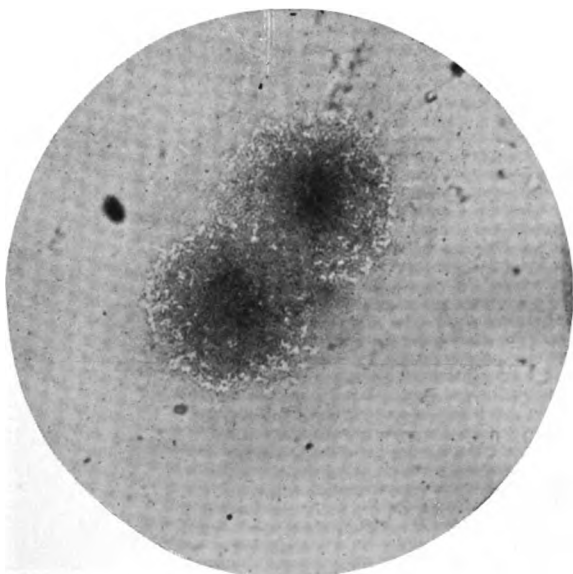


FIG. 6.

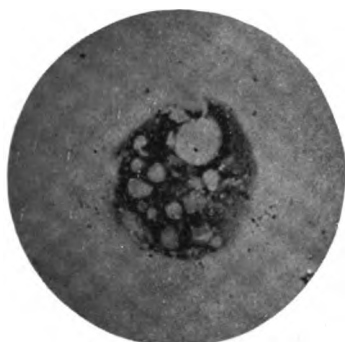


FIG. 7.

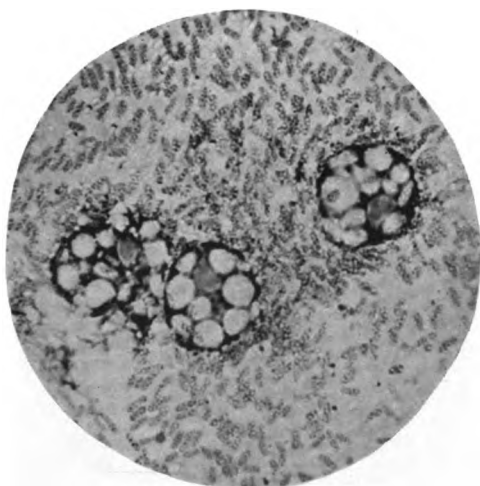


FIG. 8.

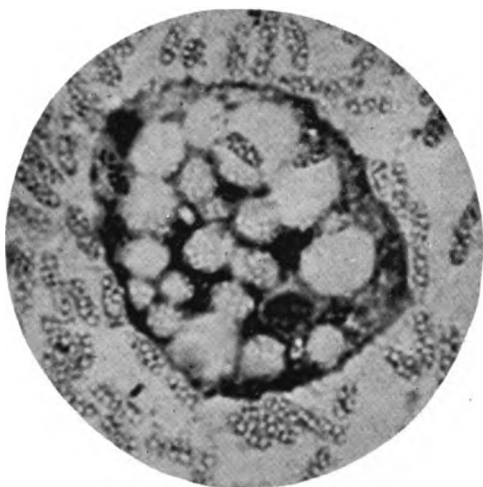


FIG. 9.

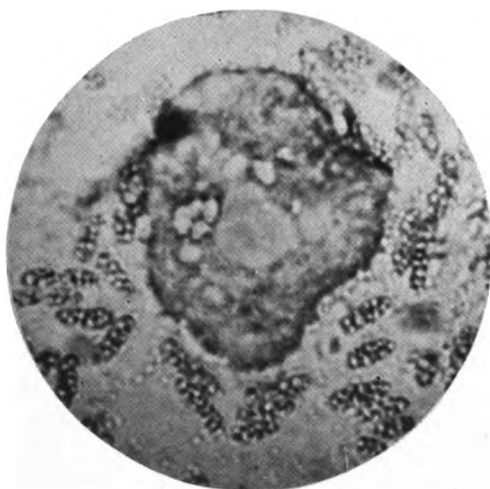


FIG. 10.

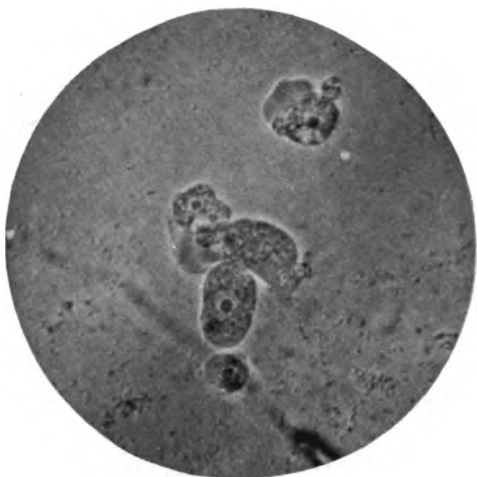


FIG. 11.

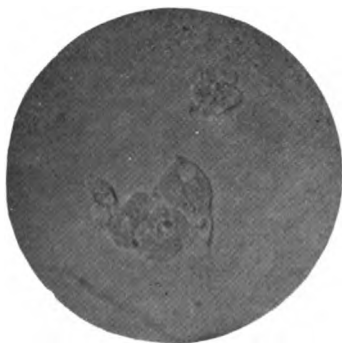


FIG. 12.

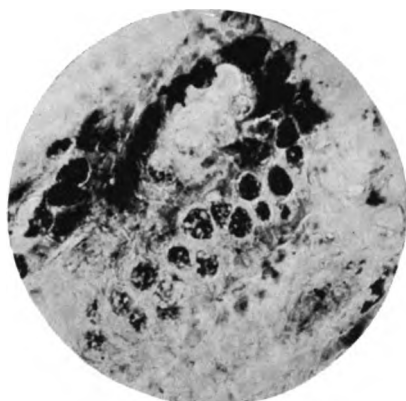


FIG. 13.



FIG. 14.



FIG. 15.

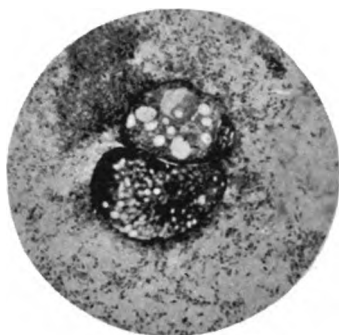


FIG. 16.

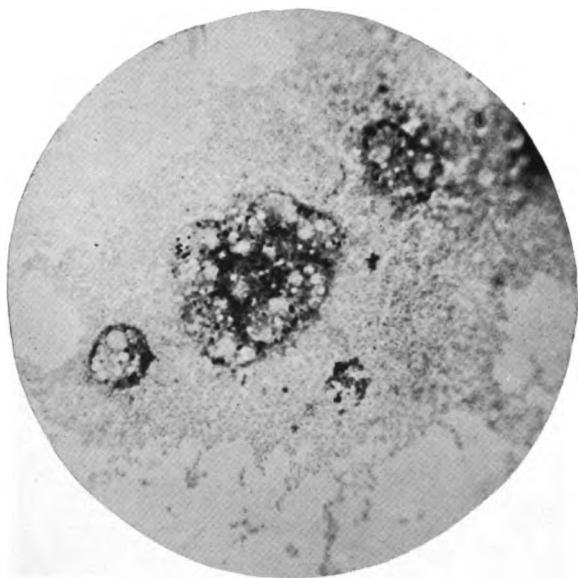


FIG. 17.

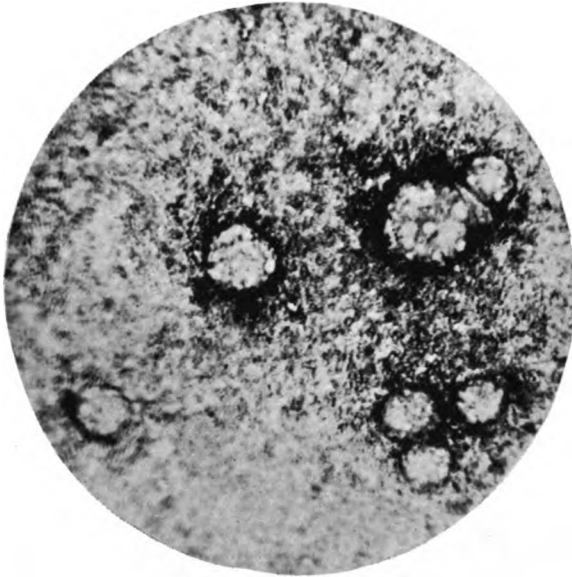


FIG. 18.

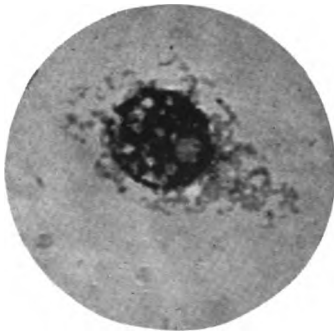


FIG. 19.

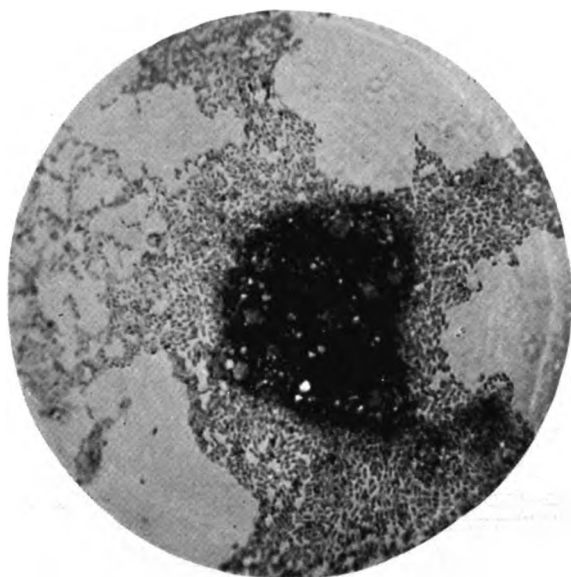


FIG. 20.

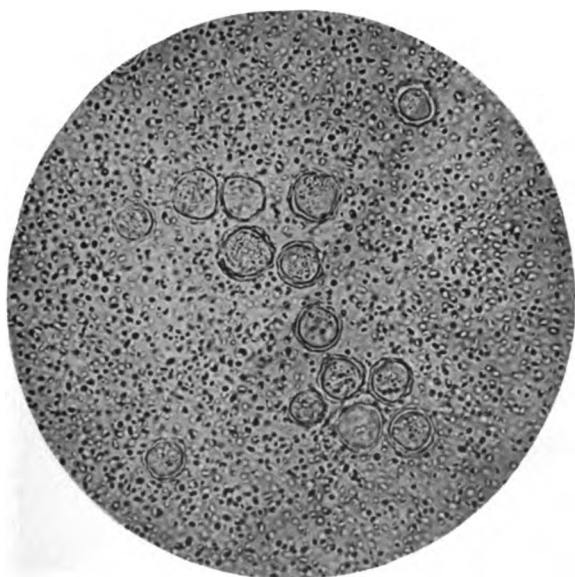


FIG. 21.

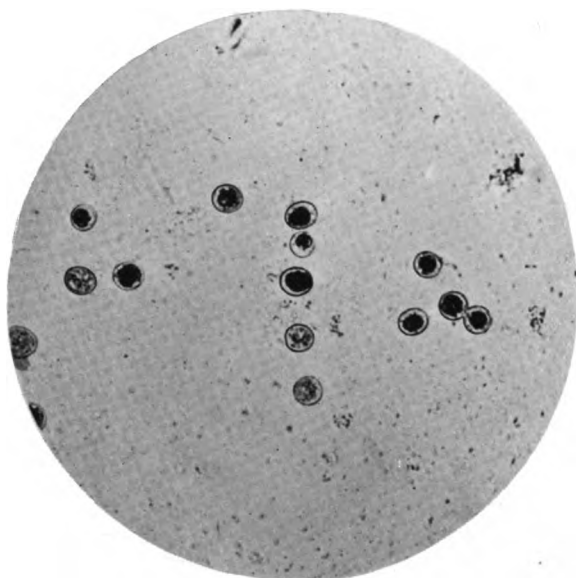


FIG. 22.

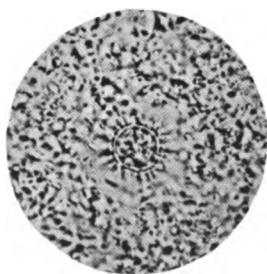


FIG. 23.

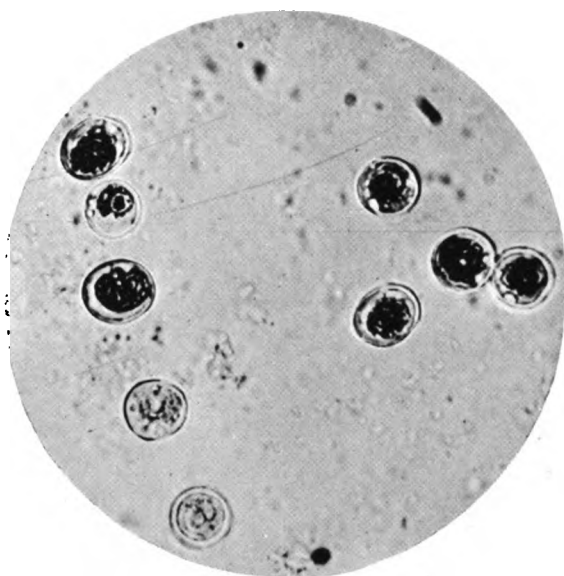


FIG. 24.

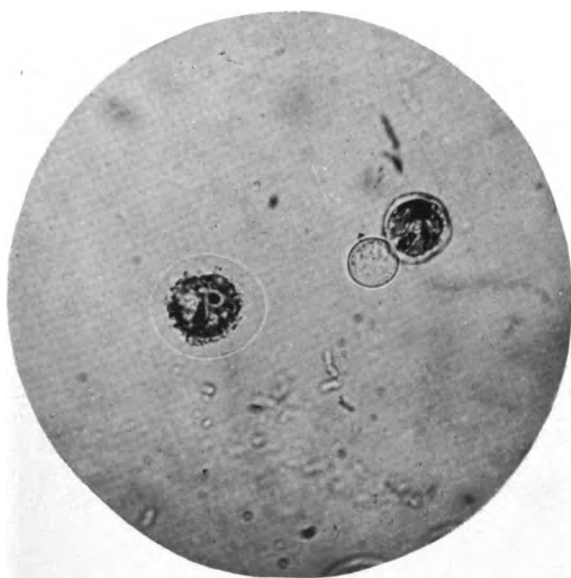


FIG. 25.

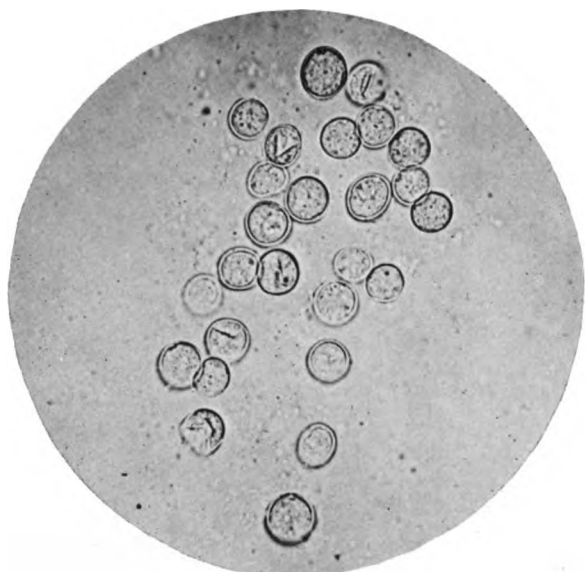


FIG. 26.



FIG. 27.

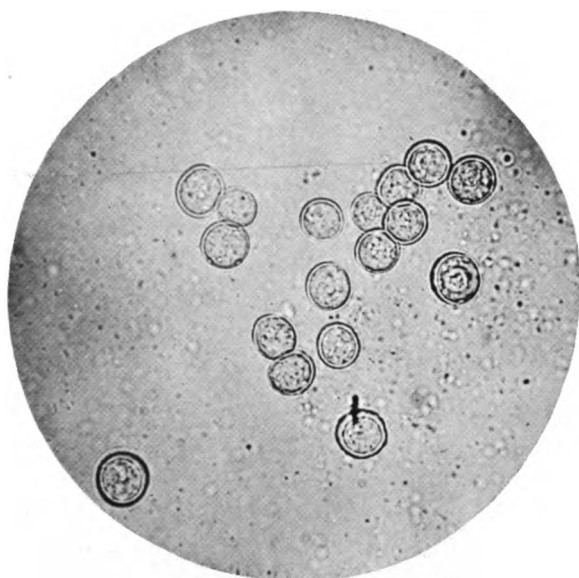


FIG. 28.



FIG. 29.



FIG. 30.

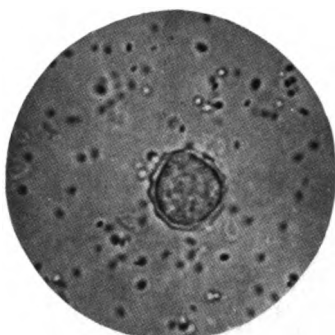


FIG. 31.

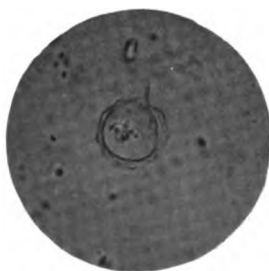


FIG. 32.

PART II.

TREATMENT OF INTESTINAL AMEBIASIS (AMEBIC
DYSENTERY) IN THE TROPICS.

By W. E. MUSGRAVE, M. D.

SYNOPSIS.

I. Introduction.

II. Prophylaxis.

- (a) Water for drinking purposes, kitchen and pantry, toilet and bath; ices and ice water.
- (b) Vegetables and fruits.
- (c) Soils.
- (d) Personal factors.
- (e) Public health.

III. Treatment.

- (a) General remarks.
- (b) Diet.
- (c) Change of climate.
- (d) Drugs and chemicals.
- (e) Local.

IV. Appendicular amebiasis (amebic appendicitis).

Pathology; classification.

Symptomatology; treatment.

PART II.

TREATMENT OF INTESTINAL AMEBIASIS (AMEBIC DYSENTERY) IN THE TROPICS.

By W. E. MUSGRAVE, M. D.

I. INTRODUCTION.

The happiest results which may follow any research are those which lend themselves to their practical application in the prevention or cure of disease, and nearly all of the great advances made in therapeutics are of this class.

Because the progress made in the treatment of intestinal amebiasis since its identification as an independent disease by Osler, Councilman, and Lafleur has been of an empirical nature, there has been a long delay in introducing the methods most generally employed at the present time, and these are still far from satisfactory. The differences which exist regarding the treatment of the disease in various places within the geographic belt where it is endemic, have also, to a certain extent, been brought about by the question as to the rôle which the amebas play in its causation. Heretofore it has not been possible to be certain in regard to this because of the failure to cultivate the parasite, but now that this is made a practicable procedure and the etiologic rôle of amebas is established, the first essential to building up a rational therapeutics is fulfilled.

Formerly, owing to failure to cultivate amebas and therefore without definite knowledge of the distribution of pathogenic ones in nature, rules for prevention have been formulated without

specific data in regard to this particular disease, and the result has been a greater lack of uniformity and success in the use of prophylactic measures than in the treatment of the infection. Inefficacy on one hand, and unnecessary severity on the other, have most certainly been the result of this ignorance.

In Part I of this report some of the important questions relating to prevention and cure of amebiasis have been elucidated, and methods and lines of work opened which will undoubtedly furnish other important data. However, this work is not complete, and a full discussion of methods of prevention and treatment, according to our present knowledge, will probably necessitate changes in the near future regarding some questions which are as yet indefinite or unexplained.

Therefore this paper will deal with the more important and heretofore neglected points in prophylaxis and treatment, and an attempt will be made to utilize some of the experimental facts which have been brought out in Part I of this report.

The complication, amebic appendicitis, is really a continuation of the disease from the cecum to the appendix, and for that reason, and because of its importance in considering therapeutic measures, it will also be briefly discussed.

Intestinal amebiasis (amebic dysentery) is the medical subject of the most universal interest in the Philippine Islands. It causes more than 50 per cent of the invalidism of public servants; it must be reckoned with in the profit-and-loss accounts of business enterprises, and it enters into consideration in military movements. It numbers among its victims all ages, all classes, and all races, being most prevalent in Manila among the unacclimated adult Americans.

Theoretically, at least, it is a preventable disease, but just how far the various theories which are supposed to insure prevention are of practicable and necessary application, while still allowing some of the comforts of life, is the problem to be solved for successful prophylaxis against it. Heretofore the only recommendation based upon facts which could be made was that of the ideal prophylaxis which, briefly expressed, is *to take nothing into the gastro-intestinal canal which has not been sterilized by heat*.

Such a procedure, while efficient, is not always practicable, is always inconvenient, and as we learn more of the habitat and manner of life of the amebas, we find that it is not necessary in

every respect. However, it is so nearly so that all departures from it should be in the nature of specific exceptions. Previously all such exceptions were in a sense due to speculation, and their variety is responsible for many cases of the disease. There are numerous Americans who have taken advantage of less stringent measures and who have escaped the disease during four of five years' residence in Manila. Others who have apparently followed similar recommendations have fallen victims, and finally, there are numbers of foreign residents in Manila to-day who have been more than four years in the country who have not contracted dysentery and who have eaten and drunk without the slightest regard for the consequences.

The principle of natural immunity (used in its most inclusive sense) is very noticeable in this disease, but it depends upon so many conditions, it is often transient, and finally, as it can not be determined by justifiable means, any conclusions based upon observations such as are given above must be unsatisfactory. However, with methods of cultivation of amebas at our command, this problem of the simplest "technique of living" compatible with safety is a practicable one.

II. PROPHYLAXIS.

To fully grasp the importance of preventive measures it must be remembered that amebic dysentery is endemic in the Philippine Islands, and that amebas which can not be differentiated from the infecting agent are easily isolated from a surprisingly great variety of substances.

Water furnishes a prolific source of the organisms, and the majority of cases of the disease can be either directly or indirectly traced to it. Practically, all the surface waters of the Philippine Islands contain the parasites in large numbers, and all the cisterns and wells which we have examined are even more richly supplied with these and other parasites. Rain water stored in cisterns is perhaps the most dangerous.

The city water supply of Manila contains large numbers of protozoa; and amebas, not distinguishable from those found in the intestinal ulcers in dysentery, may be drawn from the tap water almost at any time, and as shown in Part I of this report, cultures of some of these amebas when ingested by monkeys produce dysentery in these animals. The danger from water has become quite

generally recognized by the public, and honest effort to overcome the difficulty is used, but the methods looking to this end are very often ill advised.

First to be considered is the use of distilled water. Much of the so-called distilled water in the city is either not distilled at all or it is stored in reservoirs which are not properly cared for, and, consequently, amebas and other parasites may be frequently cultivated from it. On the other hand, it often happens that a safe water is contaminated by handling after its receipt from a reputable source. It is taken into a vessel which has been washed in tap water, or, if this is not the case, is placed on ice in bottles which have had similar treatment. In the last few weeks we (Mr. Clegg) have cultivated amebas from the water kept in bottles for drinking purposes from five different residences, and similar results have been obtained in cultures from the water coolers in public offices. Where water is boiled for drinking purposes, similar contaminations may result from handling, and filtration should never be relied upon for preparing drinking water unless the filter and method of its use and care are thoroughly understood. The rather extensive practice of filtering water after it has been boiled is particularly pernicious. If filtration is desirable it should always be done first and the water boiled afterwards.

The use of carbonated and other bottled waters, both imported and of home manufacture, is coming more and more into general use, and but for their expense would soon solve the problem for the better class of people. Here, though, as with the distilled waters, care in the source of the supply is necessary, for many of the local bottling works issue a product of doubtful purity, and in some instances have sold such an article under the label of a more reputable firm. Such observations must also be extended to imported waters, for some of those which are at times on the market here with labels of waters known to be pure are (in reality) bottled on some of our neighboring coasts and are certainly, in certain instances, dangerous. However, perfectly safe and good bottled waters, both domestic and imported, are on the market.

The question of the judicious use of water in the kitchen and pantry is an important one, although satisfactory recommendations are difficult. In the Tropics the housewife does not feel inclined to spend time in the kitchen, and servants are not trustworthy. The average kitchen in Manila is dirty, and is supplied

with tap water which usually is used by the servants in preparing foods and cleaning all dishes and vessels. From what has already been said, the danger from such sources is obvious, and the correction is practicable and not very expensive. Every kitchen range could well be supplied with a large hot-water tank, such as is commonly used in America, and this tank should be the only source of water for both kitchen and pantry. An ordinance requiring the installation of such a boiler as a part of the plumbing system of houses of the better class would do something toward controlling a disease which causes more invalidism than some of the other more prominent infectious diseases for the control of which large sums are annually spent by our Government.

Water used for the mouth should, of course, receive the same care as that for drinking purposes. The city water is satisfactory for the bath, provided care is exercised to avoid its introduction into the mouth or rectum. Such water, of course, should never be used either for the vaginal douche or as a simple enema. Both these practices are suprisingly common and many cases of dysentery are undoubtedly brought about by the use of the latter.

In Part I we (Mr. Clegg) have shown that amebas are much more dangerous when in the encysted stage, and that, among other causes, cold will always cause the parasites to assume this condition. From this it is obvious that water, as well as other articles of diet which contain amebas, is more apt to cause infection when taken cold. Of the substances served in this way, the ices and ice cream deserve especial notice. It is perfectly certain that much of the "sorbete" sold on the streets contains encysted amebas, and careful analysis would probably show the impurity of some ices from more reputable sources. These are standard preparations for social functions, where they are served in large quantities under abnormal conditions, and hence increased elements of danger of infection are always perfectly apparent. After one of the large receptions given last autumn we cultivated dysenteric amebas from the washings from the ice-cream dishes and from the water in which they were washed, while samples of the ice cream taken from the original container were free from them.

The surfaces of uncooked green vegetables and fruits are another frequent habitat of amebas and a common means of the transmission of the parasite to the human intestine. Since the foreign population has become educated to the point where they use drink-

ing water of a quality which is, as a rule, safe, the use of green vegetables is probably one of the most fertile of the remaining sources of infection, and the number of people, otherwise reasonably careful, who will eat these things with impunity without any special preparation, or at most, after they have been rinsed in distilled water, is perfectly surprising. Others who would not risk such a course will allow their use in garnishing foods, or in flavoring soups, which is accompanied by almost as great a risk. Lettuce, one of the most frequently infected of vegetables, was formerly not used by the more intelligent class of people, but recently it has become possible to obtain a nice-looking quality of this article at the Agricultural Experiment Farm, and it has become quite a popular article of diet.

On February 9 we (Mr. Clegg) obtained some lettuce from this farm with a view of determining the presence or absence of amebas, both before and after washing the leaves. One head was placed in a large sterile flask, a quantity of distilled water added, and the whole thoroughly shaken for several minutes. The water was then carefully drained into another sterile flask, and, without removing the lettuce from the original container, it was submitted to four additional washings, any one of which was more thorough than would be given by a careful housewife. Tubes of fluid from each of these washings were then centrifuged, and a microscopic examination showed amebas in two of them. Cultures were made from all these flasks and amebas which, as has been shown in Part I, were those of true amebic dysentery, grew in every tube, although they were most numerous in the first washing.

Nearly all other vegetables and some fruits yield results similar to that which we obtained with lettuce; the fruits with thick, sound skins and those which are strongly acid are *probably* safe articles of diet, but it must not be forgotten that one of the first amebas ever cultivated was from sour, fermenting grapes. Boiling water kills amebas almost instantly, and there are but few fruits or vegetables which are damaged materially by scalding, provided they are first made very cold by placing them for a time on ice.

Soil is a natural habitat of amebas, and the organisms in such a location are particularly numerous in the Philippine Islands. From such a source water which might otherwise contain but few amebas is often richly supplied. This is particularly shown by the almost epidemic spread of the disease following the recent

flooding of Manila and which brought with it the washing out of cesspools and other places where otherwise the parasites might have remained harmless. Methods of personal cleanliness and other necessary precautions to avoid the introduction of amebas from such sources into the alimentary canal suggest themselves.

Alcohol, as in many other diseases, has received a great deal of discussion as a preventive agent in dysentery. It undoubtedly exerts a destructive action, even in moderately dilute solution, but that its habitual use is of value in preventing infection is not proved. The acidity of the normal stomach probably plays an important preventive rôle, and, while hyperacidity may follow the use of alcohol, that condition is not necessarily constant.

There are certain important personal factors which enter into a consideration of prophylaxis. Children are less susceptible to the disease, and in them it is more amenable to treatment. This is particularly fortunate when their habits and the difficulty with which they are controlled are considered. The unacclimated Caucasian is the most liable to contract amebiasis, and the newcomer should always secure proper advice in order to escape preventable consequences.

One should strive by exercise and, in general, by living a well-regulated life, to keep in good physical condition; lowered vitality from any cause, diarrhea, indigestion, constipation, and many other personal factors certainly predispose to the infection.

Amebas, not distinguishable from those in the intestinal contents in amebic dysentery, have been found present in the mouths of healthy people, and it behooves residents of the Tropics to give scrupulous care to these exposed mucous membranes by frequent cleansing with distilled water or some mild antiseptic.

The normal acidity of the stomach is probably one of the preventives to the entrance of living amebas into the alkaline intestine, and its derangement, which is so frequent in warm countries, is increased materially by fermentation and other abnormal conditions in the mouth. The hands are so often in contact with materials containing amebas, and in many instances are used in the preparation and serving of food, that their cleanliness always should be a consideration. Above all, *methods of prevention must not be used spasmodically*. Safety depends upon constant and consistent adherence to a well-defined plan of living, and no

departures from it, even in minute details, should be made without securing the approval of competent authority. Among the better classes, the theoretical outline of living is generally very good, but a violation in the details of its consistent execution is extremely common and flagrant. Examples of this might be cited almost indefinitely, and the manner of infection is more often traced to such lapses in daily practice rather than to a fault with the general plan of living.

The medical profession is partly to blame for all these things. Our first duties are to guard the public health, and in the Philippine Islands there is certainly no other one thing so important as to teach the public a proper technique of living.

Finally, I wish again to emphasize that amebiasis is an infectious disease, and to point out the desirability of judicious municipal ordinances and regulations looking to its control. I hardly need call attention to the fact that its ravages do more to cripple Government and private enterprise of all kinds and conduce more to personal dissatisfaction with the country than do those of all other infectious diseases combined.

The control of cases of amebic dysentery by public-health officers is neither necessary nor desirable, but regulations requiring the report of every case with certain data would accomplish good in several ways. Valuable facts for future work could be obtained, and, what is more important, such a procedure would particularly call forth public attention and interest and would prepare public opinion for coöperation and support in controlling the disease.

For several years the military have been improving regulations looking particularly to the prevention of this disease, and results of these precautions are shown in the following figures:¹ During the month of July 439 patients drawn from approximately 2,742 troops, were treated at the First Reserve Hospital, and of this number 9 were suffering with amebiasis.

During the same month 318 patients from approximately 2,500 civil employees were treated at the Civil Hospital and by private physicians, and of this number 90 were suffering from amebiasis.

¹The figures used here have been furnished by Maj. J. M. Bannister, surgeon, U. S. A., commanding officer First Reserve Hospital, Manila, by Dr. H. E. Stafford, Attending Physician and Surgeon, civil officers and employees, and by a number of other physicians in Manila. My thanks are due to these gentlemen for their courtesy.

During the month of July, therefore, over 3 per cent of 2,500 civil employees suffered from amebic dysentery while but three-tenths per cent of 2,742 soldiers were treated for the same trouble.

There are business houses here whose employees live according to sanitary rules laid down by a physician, and such a practice should extend to civil employees and be more general among the public.

A civil employee arriving here from the United States gains his ideas of how to live from lay sources, and in the military he receives his information from published regulations. Provision by which employees of the Civil Government could receive proper instruction in the elements of prevention would amply repay such efforts by increased efficiency, just as it has proven to be the case in both the military and in private enterprises.

III. TREATMENT.

General.—Successful attempts in the classification of dysenteries from an etiologic standpoint are so recent that the great majority of the literature relating to the treatment is of but little value in its application to intestinal amebiasis (amebic dysentery), which is now a generally recognized etiologic clinical and pathologic entity—a disease different from other forms of intestinal flux in many essential particulars.

In general, it may be said that one form of treatment, in Manila at least, is now quite commonly in use, but the tendency is too much to routine. I wish to emphasize in the beginning that *any routine treatment* of this disease will prove unsatisfactory in a large number of cases. There is no disease with which we have to deal which requires more careful study of individual cases and greater variation, within limits, in the manner of applying remedial measures.

Before instituting treatment of any kind the patient should have a careful physical examination, and the history should include, particularly, efforts to specifically locate the manner of infection. Palpation of the abdomen, in connection with the history, will usually furnish considerable evidence of the duration of the disease, and what is more important, the location of the lesions in the bowel. A weekly record of the weight will always be of service, and is one of the best indications of progress.

The treatment of the patient should be governed somewhat by the findings made at the physical examination, by the clinical mani-

festations, which are so varied in this infection, and by the probable duration of the disease at the time the patient comes under observation.

Acute dysenteric manifestations, whether they come on early in the disease (which is rare) or as an exacerbation in cases of some standing (which is frequent), require the same general measures as those applicable to acute colitis from other causes. The patient should be confined to the bed, and should be required to use the bed pan; the diet should be fluid, in the form of peptonized milk, pressed meat juice, whites of eggs beaten up with soda water, lime juice and soda, etc. The pain may be controlled by local applications or by opium which can be given internally in the form of Dover's powder or paregoric, or subcutaneously as morphine. Calomel and salines, and sedative or cold-water enemas are also useful.

After the subsidence of the acute symptoms and in all other cases, the general measures should be much less severe. It rarely is necessary or even desirable to confine patients to the bed, and in the great majority of cases, in the absence of decided dysenteric manifestations or symptoms of some complication, carefully directed exercise exerts a most beneficial influence.

It would be found a good rule to confine all patients to their rooms during the first few days of the treatment because of some of its disagreeable features, and exposure to the hot sun is, of course, to be avoided at all times. In the early evenings and early mornings, however, a drive will often accomplish more good than many doses of tonic medication, and where patients have comfortable home surroundings it will rarely be necessary or advisable to send them to a hospital.

Where emaciation and anemia are advanced, particular attention must be paid to building up the general health, and with well-nourished patients, treated early in the disease, care must be used to maintain this condition during the more or less lengthy and trying period of active local treatment.

Diet.—Notwithstanding the possible presence of extensive ulceration, the appetite and digestion often remain good, and when we consider the local nature of the disease and that, excepting complications, death frequently results from asthenia, it is readily seen how urgently necessary is a sufficient amount of nutrition.

The tendency, at least in Manila, has been to unnecessarily limit the diet in the ordinary type of cases, but the greatest reason for any such restriction is to limit the mass of fecal matter in the colon, where it may interfere somewhat with the efficacy of local treatment. When the stomach and small intestine are deranged, and sometimes when this is not the case but where clinical manifestations show involvement of the cecum or upper colon, a selected diet is indicated; and when the rectum is very irritable such a restriction quite frequently appears to increase the tolerance for the enemas. But, in the more usual type of cases, there is little evidence that a liberal diet interferes in any way with the efficacy of the local treatment—in fact, quite the contrary often may be observed. In some of the more advanced infections, where emaciation and anemia are progressive, and where, as is often the case, the patient has no desire for the usual liquids, etc., a carefully prepared beefsteak will be relished and borne with comfort. The amount of residue in such instances does not need to be considered, for it may be disposed of by a simple enema given a half hour before the usual local medication, a procedure which, in any event, is often useful.

It has come to be my custom in cases of the ordinary type, and in all where severe clinical manifestations have subsided, to allow a very liberal diet; forbidding only irritating foods and such as are likely to ferment and those leaving a large bowel residue. In the class of cases where the cecum alone or the cecum and ascending colon are involved, the clinical manifestations are often very slight, and extensive ulceration may be present before the nature of the disease is suspected. Treatment of these is difficult because of the care necessary to reach the lesions by enemas and because of the soft fecal matter usually clinging to the mucous membrane in this part of the bowel. A fluid diet sometimes makes a material difference in the treatment, but even here an occasional saline cathartic or a large simple enema once or twice daily will usually accomplish more and at less expense to the patient's strength than a reduction in the amount of food.

Change of climate is an important factor in treatment. Alone, it is not a specific therapeutic agent in any sense of the word, but it is an especially valuable aid, particularly in old, emaciated cases, and should be employed, where possible, with all patients who do not react to treatment in local environment. However, except

under extreme necessity, it should never be recommended nor patients allowed to pass from observation without first having a course of local treatment, and great caution should be exercised in advising a change to those who have symptoms of some of the more common complications. In nearly all cases the improvement is but temporary, unless treatment is continued, and for that reason patients should have specific directions to consult a physician upon arrival at their destination, and, where practicable, letters should be given explaining the condition.

Drugs.—The list of drugs which have been used in dysentery and vaunted from time to time, many of them as specific, is too long even to mention, and, so far as they may have any *specific* curative properties in the type of the disease which is under discussion, they are all useless.

It requires no very extensive observation to show that this practice of drug giving is a much-abused one, and the actual harm done by it is not inconsiderable. There was a time in this country when such things were excusable, but with the knowledge obtained by experience and research their continuation as routine treatment deserves the severest condemnation.

There are a few drugs which, on account of their very extensive use, deserve special consideration. First, the various salts of bismuth, particularly the subnitrate and subgallate, are both useful therapeutic agents within limits, but their abuse in the treatment of amebiasis is very great. In the absence of the more rational local treatment, in combination with some of the other internal remedies, bismuth may be a very useful drug, and at other times is probably very nearly harmless, but when given at the same time quinine enemas are being used it undoubtedly does do harm.

Observations made at necropsy furnished abundance of evidence in support of this statement. As is well known, these salts are insoluble in the intestinal canal, and so their action is largely mechanical. In a bowel which has been ulcerated for some time, they impregnate the edges of the ulcers and all other more or less dead tissue to such an extent that at post-mortem, even after the most thorough washing, enough is often left to make the surface almost black. The coating formed is so tenacious and fastens itself so firmly about the ulcers that quinine and other curative substances applied locally have little opportunity to reach the most essential places. There is also ample clinical evidence

corroborating these statements. I have seen patients who have taken bismuth internally together with quinine enemas for considerable periods of time, while amebas were constantly present in the stools but, on substituting small doses of a saline for the bismuth, a permanent disappearance of amebas occurred within a comparatively short time. The more usual and equally tenable arguments against the indiscriminate use of this drug need not be entered into. The important consideration which may be amply demonstrated is that the drug is not harmless, as it is popularly believed to be, but, during the time of local treatment, it is capable of doing much damage in a negative way if in no other.

Much of the abundant literature about the use of ipecac is valueless because of the lack of sufficient clearness as to the type of the disease referred to by the writers who advocate the drug. Whatever may be its value in certain other forms of dysentery, it is quite certain that when given in the doses and the manner prescribed by its most ardent adherents, it is not only useless, but may be dangerous in amebic infections of long standing. In small doses it undoubtedly acts as a tonic to mucus surfaces, if nothing more, and as such often may be administered with advantage in intestinal flux. In emaciated patients who have had the disease for a long time, and when given in the large doses which are usually recommended, its results are sometimes disastrous. I have seen three cases (with post-mortem) in which, in my opinion, it was the immediate cause of death. These were patients who should have remained alive for weeks, unless complications had developed, and one of them might have recovered under more rational therapy.

Magnesium sulphate and other salines have been much used in the treatment of dysentery, and where a cathartic is indicated in well-nourished patients they are very satisfactory, but their routine use for considerable periods of time, particularly in patients in the more advanced stages of the disease, is not to be recommended. They have, at least, two specific actions—one is to increase the alkalinity of the intestinal contents and thus favor the propagation of amebas; the other is to cleanse the mucus membrane and thereby allow greater efficiency of the enema. Further than when indicated as an active cathartic for this latter action, they should be used in this disease with caution.

There is some reason for the use of the mineral acids, especially hydrochloric and nitro-muriatic, and the efficiency of hydrochloric acid is increased almost always by its combination with pepsin; sometimes other digestive ferments are useful. In the vast majority of the usual cases, the acid-pepsin solution has at least two favorable actions—first, that due to its acidity alone, and second, it is particularly active in reducing or preventing the nausea which is often a troublesome sequence to enemas, which condition is principally due to reversed peristalsis. For this purpose neither the pepsin nor the acid alone is as satisfactory as the combination.

The so-called intestinal antiseptics are often of service in allaying fermentation, and possibly may also limit somewhat the number of bacteria. Salol or guaiacol carbonate in combination with minute doses of ipecac often exert a good influence, and the more recent preparation—acetozone—gives good results as an adjuvant in many cases. To get the best results from acetozone it should be drunk freely in 1-5,000 to 1-2,000 solution, as much as one to three or more liters being consumed in twenty-four hours. It is much more palatable when the solution is made in carbonated water, and one of the most satisfactory methods of prescribing it is to have it carbonated in syphons or ordinary soda bottles with directions to use instead of water. Its action as an intestinal antiseptic is, like other preparations, somewhat limited, but in cases where there is active fermentation of the stomach and upper bowel it often gives good results. I have seen some unfortunate results follow the administration of celloidin-coated capsules of this drug, probably due to rupture in the intestine of imperfect capsules and the consequent liberation of the chemical in concentrated form. Its use in enemas will be considered under the local treatment.

Strychnin and other powerful stimulents should be used with care during the administration of enemas on account of their stimulating action in the bowel. Strychnin, particularly, is a valuable general tonic and stimulant, but during the employment of local treatment it is best replaced by some of the more diffusible drugs; alcohol in the form of champagne, dry sherry, or punches, is usually grateful to the patient and satisfactory in results.

Except in those cases complicated with malaria the internal administration of quinine so highly recommended by some is use-

less, and even when malaria is present the objects sought are much better obtained by the quinine enemas which, if properly administered, will result in cinchonization of the patient. Indeed, this is one of the disagreeable features of this treatment in a very large percentage of cases.

Calomel has had many advocates on account of its supposed antiseptic action in the intestine, and given in small frequent doses it may exert a beneficial influence.

I have never seen satisfactory results from the use of sulphur. In fact, as stated before, there is no internal medication which is in the least sense specific in intestinal amebiasis, and such treatment should be directed to the improvement of the general health of the patient and to the alleviation of some of the prominent symptoms as they arise. All insoluble substances which coat the bowel are contraindicated during the time that the patient is being treated by enemas.

Local treatment, properly carried out, gives satisfactory results in the largest number of cases, but to insure success constant care, close study of individual cases, and the surmounting of many obstacles are necessary.

The manner of giving the enema determines in no slight degree its efficiency. Routine is particularly dangerous, each case requiring careful consideration, and variations should be made according to the indications to be met.

The *apparatus*, in private practice, should consist of a glass irrigator of at least two liters capacity, inclosed in a metal frame, and in hospitals the larger adjustable glass irrigators meet the requirements particularly well. Rubber bags are never satisfactory for reasons obvious to those of experience in giving these enemas. The tube should be of very soft rubber five or six feet long and connected by a valve stopcock with the rectal tube, which in turn should be at least 100 centimeters in length, from 10 to 15 millimeters in diameter, and made of the best red rubber. It should be of moderate firmness, not so stiff as to be dangerous to the ulcerated bowel nor so soft as to easily fold upon itself during introduction.

Contrary to what is often recommended, the opening should be in the end of the tube, well rounded, and the immediate extremity slightly contracted and hardened. The disadvantage of having the opening in the end has been said to be that it is

much more likely to become closed by fecal matter or pressure; but, in my experience, this has not been the case. Such a rectal tube will occasionally become clogged, but very rarely so when judgment is used in its introduction. The weakest point of this tube is its extreme end, and if any obstruction occurs during its entrance with the current flowing (as it should be under low pressure) it is quickly observed by a lack of pulsation in the soft delivery tube (which should always be under control of the operator's fingers), or by watching the fall of fluid in the irrigating tank, and such trouble always may be quickly corrected by withdrawing the tube very slightly and waiting a few seconds until the fluid, which should be allowed to escape in a pulsating manner from the delivery tube by alternating compression and relaxation, has removed the obstruction by dilatation of the bowel. If the tube opens both in the end and on the side the means of detecting an occlusion are the same as with the other. However, on account of the two openings, when the fall of fluid indicates a stoppage the tube already may have been folded. This trouble usually begins at the eye, and several feet of loop may have been introduced before both openings are closed and the flow is stopped. In this case a correction of the trouble necessitates a further withdrawal and the turning of a knuckle of an inch or more of tube in the ulcerated bowel. Finally, with the tube opening at the end there is obviously much less danger of mechanical injury to any deep ulcers which may be present. The tube with an opening on the side alone is, of course, not to be considered.

A very good position for the patient during the administration of an enema, and one which is usually most convenient, is the Sims position, with the hips well elevated. When practicable, it is still better to have the foot of the bed raised from 12 to 18 inches. The knee-chest position on the same kind of a bed is sometimes particularly satisfactory, both because of the ease in passing the tube and the ability to retain the fluid. With the rectal tube and anus well lubricated with clean castile or other mild soap or soft white vaseline, the tube, freed from air, is introduced through the sphincter. After a slight rest for the spasm of the sphincter to subside, the flow may be turned on under low pressure (controlled by the fingers on the delivery tube) and the latter is then slowly introduced to a distance of from 40 centimeters to 1 meter or more, depending upon the character of the case, the location of the

lesions, and the patient's tolerance for the fluid. After the tube has been introduced to the highest point which is necessary the rate of flow may be increased and the amount of fluid to be introduced is governed by the bowel capacity or the patient's ability to bear the pain, which in some cases is excruciating.

Patients often will be found who will take from 3 to 4 liters with no great discomfort, but in the majority about 2 liters will be all which can be borne, and in some, not over a liter is practicable. Naturally this variation in quantity depends upon the differences in bowel capacity, upon its irritability, and upon the patient's idiosyncrasy to pain. Women, as a rule, are more satisfactory patients in this respect than men.

Several other factors help in introducing a large quantity of fluid into the bowel, but they can not be determined always without experiment. Bowel spasm comes on in nearly all cases during the entrance of the fluid. In some instances increased pressure gives the best results, while in others a decrease in, or a temporary stopping of the flow, will be found most satisfactory. Often allowing a patient to change position during the flow will give relief, and if this is done with caution there is no objection to it. Occasionally a bowel is so irritable as to preclude the giving of an enema of sufficient concentration and quantity. This difficulty may be greatly relieved by giving one of warm water or salt solution containing one-fourth to one-half grain of morphin a half hour before the medicated enema is introduced.

The presence of gas in the bowel always increases the pain caused by the enema, and hence, in those cases where there is considerable fermentation or where the bowel has a large accumulation of feces, it is often well to give a preliminary simple one. The latter cause little or no pain, they cleanse the bowel, and in that way allow increased certainty of action of the medicine to follow. Their use gives such happy results in many cases and their inconvenience is so small that I have employed them very frequently as routine, even where there are no special considerations to be met.

After a full enema has been administered, a change of position usually enables the patient to retain it longer, and in addition may insure its reaching all parts of the colon. If the sloping bed has been used it will usually suffice for the patient to turn on the back with legs flexed, or on the right side, and if further

elevation is desired it can best be accomplished by still further raising the foot of the couch.

In every case all practical methods should be applied to give the largest quantity of fluid which can be administered and retained, for to secure satisfactory results the diseased part of the bowel, which in the majority of cases is clinically the entire colon, must be distended and the fluid retained from five to fifteen minutes. Retention of the enema for less than five minutes hardly allows it to develop its full parasitocidal action, and when the fluid is held for more than fifteen minutes the result may be unnecessary inconveniences from the absorption of quinine. The latter is a very troublesome condition in very many cases.

The temperature of the solution not infrequently makes a material difference in its tolerance. It has been shown that the amebas when they are in the moving stage are most susceptible to the action of antiparasitocidal agents. This condition is interfered with by either cold or heat. For this reason cold solutions would appear at a glance, at least theoretically, contraindicated either as a vehicle for the curative medicine or as sedative applications immediately preceding the medicinal ones.

There is, however, another factor to be considered here, for while the cold increases the parasite's resistance to medicinal agents, it also, by causing it to encyst, removes its power of independent motion and its ability to cling to its surroundings and therefore it may be much easier to flush from the bowel.

Tuttle has recently reported good results in the treatment of amebic dysentery by the use of simple ice water enemas given frequently, and his success may be explained by mechanical removal of the parasite.

Cold solutions, while quite disagreeable during their introduction, later often have such a soothing influence on the mucus membrane and as a consequence for some time, when other remedies fail to create tolerance in the bowel, I have often used an ice-water injection one-half hour before the quinine enema, and sometimes have administered the quinine itself in cold solution.

After Tuttle's article more attention has been paid to this point. However, it must be remembered that the lowered temperature produced by cold injections in the bowel is only temporary, and that those amebas which are not flushed out during this time are not destroyed, and their progress is but slightly delayed.

In order to secure whatever advantage might be attributed to the cold and at the same time to introduce the antiparasiticide agent, a few old cases which were very resistant to treatment were placed on ice-cold solutions of quinine instead of the usual ones at the body temperature. This method has been used with about twenty persons in private practice, mostly among those who have not reacted well to the usual treatment. The results so far, while encouraging, have not been universally satisfactory.

Such ice-cold quinine enemas are often quite painful when they are first introduced, but this condition is only a transient one and is frequently followed by a sense of comfort rarely experienced when the solutions have a higher temperature. There is some local reaction, of course, but I have never seen any dangerous manifestations. It is much easier to reach the upper colon with such an enema, and this fact alone makes it useful in many cases.

Having discussed the technique of the administration of enemas we will now consider more in detail some of the therapeutic agents to be used.

A considerable variety of drugs from time to time have been recommended, but up to the present time the various salts of quinine have given the most satisfactory results and have been the most constantly and extensively used since their introduction by Löscher in 1875.

If enemas of this drug are properly prepared and administered the outcome in the majority of cases of moderate duration is very satisfactory, and many of the disappointing results following its use are not the fault of the principle but the result of routine and the failure to use it under the most favorable conditions.

There is apparently but little choice between the various salts of quinine, but to be most serviceable the solution should be an acid one. The strength used should vary from 1-1,500 to 1-750, depending somewhat upon the idiosyncracies of the patient. A stronger solution than 1-750 is not required to destroy any amebas which are subject to its action, and besides it is more irritating, and when retained produces unnecessary systemic effects from the absorption of the drug. A weaker one than 1-1,500 does not assure parasiticide action in a reasonable length of time, and is therefore not to be used.

The fluid used as a vehicle for the application of the quinine also deserves some thought. In 1884 Nothnagel showed that when sodium

chloride was placed upon the peritoneal coat of the living intestine, active antiperistalsis resulted. In 1894, Grützner injected into the rectum physiologic salt solution in which was suspended finely divided hair, charcoal, and starch. After six hours he demonstrated these substances in the small intestine and stomach. Hemmeter (1902) confirmed these observations, proving that active antiperistalsis followed the introduction of substances suspended in salt solution into the rectum of human subjects, a cat, and a white rat. He further showed that these results were less certain or not to be obtained when solutions of potassium chloride or hydrochloric acid were used. Fasting favored this antiperistaltic action and both diarrhea and constipation delayed it; the normal bowel gave the best results. Peristalsis was *not* arrested by this procedure, but continued to control substances well within the lumen of the bowel, while the antiperistaltic movements influenced only substances near the walls of the organ.

Accepting these statements as proved, their intelligent application may be of service in the local treatment of amebic dysentery, particularly in the selection of a solvent for use in the application of quinine or whatever other antiparasitic substance is used in enemas.

Not infrequently one of the disagreeable features of the local treatment, whatever the medicine, is the nausea which follows and the consequent interference with digestion and nutrition. One would not think, therefore, of employing a sodium-chloride solution as a vehicle for quinine in such cases, but on the other hand, in certain well-nourished patients with decided intolerance for enemas, this condition may be taken advantage of, and if necessary a sodium-chloride solution enema containing a quarter of a grain of morphine or one-tenth of a grain of cocaine may be administered one or two hours before giving the regular quinine one.

Recent researches have tended to show that the presence of living bacteria are necessary for the propagation of amebas, and if this symbiosis is in reality a necessity to the life of the amebas in the intestine we have an increased incentive to use bactericidal local medication. Bacteria are quite generally considered as having pathologic significance in this disease, partly, no doubt, as a result of their own action and in addition, the symbiosis referred to may result in metabolic changes which may vary with different bacteria and influence the pathologic action both of themselves and of the parasites. Whatever the manner of their action, the clinical importance of limiting the number of intestinal bacteria in this infection is fully recognized. Various kinds of antiseptic

irrigations looking to this end have been used and recommended, but in satisfactory concentration they have usually proven either dangerous or impracticable.

In 1902 Freer called attention to the results obtained by Strong in the experimental treatment of eleven cases of amebic dysentery with benzoyl-acetyl peroxide (acetozone). The drug was given both internally and by enema. In these cases the results were encouraging. Of the eleven cases treated, two died, one from a complication of a liver abscess. The other nine at the time of the report were without any symptom of the disease.

The bactericidal properties of this preparation are well known, and its action in acid solution on amebas has been pointed out in Part I of this report. I have never used it alone in treatment, but in acid solution of 1-5,000 to 1-2,000 in combination with quinine 1-1,500 to 1-750, or when used alternately with the quinine enemas, it often gives more satisfactory results than does quinine alone in cases in which it is tolerated.

The inconvenience in securing the solution for private practice and its irritating effects in the colon are the principal objections to it, and the latter is partially overcome when it is used in the cold enemas described above.

Succinic peroxide acid ("alphozone") used in the same manner as acetozone has recently given some encouraging results, and in some very chronic cases the happiest results sometimes follow the occasional substitution of an enema of 1-10 to 1-2 solution of hydrogen peroxide for the quinine one. In a like manner an occasional reaction brought about by an injection of a solution of silver nitrate does good, but the routine use of this drug is not usually satisfactory.

As to the number of enemas to be used, the tendency is to overdo. One enema properly administered is of more value than several given without consideration of the conditions. In the vast majority of cases one to three in the twenty-four hours will be found to be the most satisfactory, and, fortunately, this number fulfills the rational as well as the practical requirements.

Some of the most aggravated cases are those in which a catarrh of the lower bowel has been established by the previous use of enemas too frequently given, of too great a concentration, and which have been introduced into the lower colon in quantities of one liter or less. In not a few cases, owing to the physiological

action of absorbed quinine or from other causes, it will be found impracticable to give more than one enema daily.

Irritability of the anus and rectum usually may be avoided, but sometimes this condition occurs and it may prove very annoying. After each treatment the external parts should be washed with soap and water and bathed in some antiseptic solution, and when the irritation becomes excessive a cocaine suppository administered just previous to the enema will afford relief, but where possible this is to be avoided, for, in addition to reasons which are obvious, it may interfere with the action of the sphincter to such an extent as to allow the escape of fluid during the operation. Flushing the lower bowel with cold water or some mild, soothing lotion after each treatment gives the most satisfactory results in these cases.

In many of the text-books caution regarding the maximum pressure to be used in the administration of enemas is enjoined in those cases showing advanced ulceration. Theoretically this is good advice, but in actual practice, in the first place, it is impossible to determine the extent of intestinal ulceration in amebic dysentery, and secondly, agreeing with Professor Osler, I must say that the danger from this source is small indeed—so much so that it is doubtful if it deserves consideration in the treatment of a disease where distension of the bowel is so necessary. In over five years' observation in hospital wards, in private practice, and in the morgue, I have never seen evidence of such an accident.

Finally, I wish to emphasize that *no* recommended routine treatment will be found to be satisfactory in all cases of intestinal amebiasis. Each one requires careful study, and variations in the treatment should be made to suit special conditions, and to efficiently do this the physician should either himself administer or be present at the administration of at least the first few enemas given to every patient under his care.

IV. APPENDICIAL AMEBIASIS.

Involvement of the appendix in amebic dysentery, as previously stated, is really a direct continuation of the diseased process from the intestines and therefore, may, be appropriately considered here.

In 100 necropsies on this disease the appendix contained lesions in 14. In 6 the process was an active amebic infection, four times in otherwise apparently healthy appendices, and in one the amebic process was secondary to chronic changes from other causes.

One was an old chronic appendicitis (nonamebic) associated with a pericecal abscess (amebic) and 7 others were those showing chronic lesions without amebic infection. Four of these seven, however, showed evidence of some acute inflammatory reaction, probably the result of close contact with the amebic process going on in the cecum.

In 4 of the 6 amebic cases death resulted from general peritonitis following perforation, three times in the cecum and once in the descending colon. This entire series was in American soldiers, and necropsies were performed by Dr. Strong and myself, mostly during the year 1900. They were almost entirely chronic cases which gave histories of months of diarrhea, and were in the last stages of the disease when received at the First Reserve Hospital.

In the more recent study of 50 fatal cases, and in many clinical ones, particular attention has been directed to the involvement of the appendix as a complication. However, the statistical results in the fatal cases have in the main been confirmatory of those in the first series.

In every instance where the appendix showed amebic ulceration this has been as a direct continuation of and secondary to a similar process in the cecum. Peritonitis following perforation of a gangrenous amebic ulcer in the appendix occurred in 1 case, but the cecum was also gangrenous. Without lesions amebas have never been found in the appendix.

With these brief data we are better prepared to discuss the clinical side of the problem, which particularly concerns us in this paper. Where the records are complete all cases which have shown lesions of the appendix post-mortem have also given a history of clinical symptoms during life which indicated involvement of that organ. However, on the other hand, *very few of the cases which have manifested clinical symptoms of appendicitis during life have shown lesions of this organ post-mortem.*

The highest mortality in intestinal amebiasis occurs where the cecum is involved, and as appendicitis is found but rarely, if ever, in any other class, it will be seen that post-mortem examinations indicate a much higher percentage with appendicitis than actually exists when all classes of cases are considered. The very great majority of those suffering with amebiasis will not have symptoms

of appendicitis during life, and this organ will be found healthy at necropsy.

From clinical and post-mortem observations we learn that cases of amebic dysentery with symptoms of appendicitis during life may be divided into three general classes.

(1) Those in which at operation or necropsy the appendix is not found to be diseased.

(2) Those in which at operation or necropsy a chronic appendicitis from other causes and without amebic infection is found.

(3) Those in which at operation or necropsy an amebic infection of the appendix is present.

Clinically examples of the first class are very frequently met with, and sometimes at autopsy no lesions are found which would account for the symptoms during life. However, in one of my cases there was a pericecal abscess due to a perforation, and in others there was extensive ulceration of the cecum both with and without adhesions to the omentum and other structures, which accounted for the symptoms during life.

The second class is not infrequently encountered during life, and in the fatal cases there is often congestion, injection of the vessels, and other inflammatory phenomena, due to close relation to the inflamed bowel, but without an amebic process or the presence of the parasites in the appendix.

The third class is the last in order of frequency to be observed in clinical practice, but is more frequent (seven times in the 150 necropsies) in the necropsy records, because, practically, all such cases are fatal.

As previously stated, these seven infections, with one exception, occurred in apparently otherwise healthy appendices, but it is important to remember here that in three of these the cecum was also gangrenous, and death resulted from peritonitis following perforation—twice in the cecum and once in the appendix. In the remaining case the amebic infection was secondary to an appendicitis from other causes.

These statistics show that the majority of appendiceal infections are associated with the very severest involvement of the cecum, where death usually results from a perforation in or near the deeply ulcerated and often gangrenous organ, the type of case corresponding so closely to similar ones without infection of the appendix that a differentiation during life is often impossible.

Sometimes when the infection is less intense in the cecum, and the lesions in the appendix more important, the clinical picture of appendicitis is more definite and may with care be diagnosed during life.

The importance of being able to distinguish during life the three classes of cases discussed, from appendicitis due to other causes, as well as to differentiate them from each other is obvious.

As a rule no great difficulty, at least in the Tropics, will be encountered in differentiating the amebic processes located in or about the cecum from appendicitis due to other causes. In the former there is generally a history of dysentery or diarrhea, and a microscopic examination of the stools will usually show amebas, while in the latter a history of previous attacks often may be secured. The general symptoms in the amebic infection are usually less severe and differ to a certain extent from those of the latter. Fever is more commonly slight or even absent; nausea is less frequent (unless the patient is taking enemas), and vomiting is rare. Rigidity of the rectus muscle is nearly always less decided or even absent, and, while pain on deep pressure is quite constant, it is rarely of the very acute type so often seen in other forms of appendicitis. A tumor mass, as in other forms of appendicitis, is usually present, but it more often has a soft, boggy character in the region of the cecum and is rarely circumscribed, definite and low in the right iliac fossa, as in other types of appendicitis. Sometimes, however, where there is a definite appendicitis due to amebas, it may be ushered in as a sharp attack of acute pain, with some muscular rigidity, perhaps a certain degree of fever, an increase in the number of bowel movements, and a leucocytosis of from 10,000 to 20,000.

A differentiation from *each other* of the various types of amebic infections which give the general symptoms of appendicitis during life is a difficult task, and it is even impossible in many instances to say that the appendix is involved at all.

Where the cecum, whether alone or with the appendix, is the seat of dysenteric lesions, pain in the region occupied by these organs is quite a constant symptom, and it may vary from a dull aching one to that of the most acute radiating type. The acuteness of the pain depends, to some extent no doubt, upon the character of the lesions, the stage of the disease, and the general condition of the patient. In well-nourished individuals moderate

ulceration in or just below the cecum may cause considerably greater pain than similar lesions in chronic emaciated cases. Some of the most deceptive clinical pictures are encountered when the omentum becomes adherent around the cecum, ascending colon, abdominal wall, and liver. When such adhesions form in the region of the appendix considerable thickening occurs and palpation, besides confirming deceptive conclusions, is to be performed with care on account of the danger of rupture.

In the second class of cases, when a chronic adhesive appendicitis has been aggravated by close relation to the diseased cecum, the patient's history usually will show similar attacks antedating the dysentery. The symptoms, which usually come on somewhat slowly, are rarely urgent, and are frequently relieved by a saline cathartic.

We have already seen that in the third class the lesions of the appendix are but a continuation of similar ones in the cecum, and in the rare instances where they are not the clinical picture is the same as that of an appendicitis from other causes.

Treatment.—The majority of cases of amebic dysentery give no symptoms indicating involvement of the appendix during life. No amebas and no lesions are found in this organ post-mortem, and therefore treatment directed specially to the appendix is worse than useless.

When symptoms suggesting appendicitis do arise during the course of the disease the *medical* treatment is that which is applicable to appendicitis from other causes plus high large quinine enemas. Saline cathartics are particularly useful if the patient is in a condition to justify their administration. Thirty grams of Rochelle salts will often relieve, more or less permanently, the symptoms of appendicitis from this cause.

In the early stages of amebic dysentery, in the majority of cases, the appendix could be removed without immediate bad results, but the probable future of the patient must be considered, and this is not best conserved by performing an operation which, for a greater or less time, necessitates a discontinuance of rational treatment of the disease, and which uses up more or less of the strength and energy so necessary for its successful therapeutics. Such precipitate surgery has, in the past, undoubtedly determined the fatal issue in some instances.

We have shown what a variety of pathologic conditions may produce symptoms of appendicitis in intestinal ambiasis and how

difficult and often impossible it is to differentiate these during life. They are produced most frequently by lesions in the cecum without involvement of the appendix where surgical intervention is contraindicated.

Somewhat less often such symptoms follow aggravation of an existing chronic appendicitis due to other causes by the continuation of inflammation from the cecum, and nearly always without amebic involvement of the appendix. Operation is rarely found to be necessary, provided medical treatment of the disease is rationally administered.

Finally, in a small number of cases such symptoms are due to amebic involvement of the appendix as a continuation of a similar process from the cecum, and early operation is indicated.

In a minority of these, appendectomy may be satisfactorily performed, but more often the operator will find a gangrenous cecum and increase his mortality rates.

In the 150 necropsies there were two cases where operation would probably have saved or prolonged life. One of these was amebic appendicitis without extensive ulceration in the cecum, and the other a pericecal amebic abscess.

A surgeon should be called in consultation in all cases of intestinal amebiasis with symptoms suggesting appendicitis, and questions of operation decided only after careful consideration.

In closing, I wish to thank Dr. P. C. Freer, Superintendent of Government Laboratories, for his assistance in editing the manuscript.



C

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No. 19.—OCTOBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES
BIOLOGICAL LABORATORY

SOME OBSERVATIONS ON THE BIOLOGY
OF THE CHOLERA SPIRILLUM

BY
WM. B. WHERRY, M. D.

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LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, September 20, 1904.

SIR: I have the honor to transmit herewith a paper by Dr. Wm. B. Wherry of the Biological Laboratory on "Some Observations on the Biology of the Cholera Spirillum."

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY, OFFICE OF THE DIRECTOR,
Manila, August 31, 1904.

SIR: I have the honor to transmit herewith and recommend for publication "Some Observations on the Biology of the Cholera Spirillum," by Wm. B. Wherry, M. D., Bacteriologist Biological Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

Dr. PAUL C. FREER,
Superintendent Government Laboratories, Manila, P. I.

SOME OBSERVATIONS ON THE BIOLOGY OF THE CHOLERA SPIRILLUM.

By W. M. B. WHERRY, M. D., *Bacteriologist, Biological Laboratory.*

INTRODUCTION.

The following observations were made during the past year while I was engaged in some studies preliminary to and in connection with the subject of toxin production.

Such marked variations in the morphology and biochemical characters of the cholera spirillum occurred during some earlier work that it was deemed advisable to adopt a modification of the methods of standardizing culture media, published as the "Procedures," etc., by the Committee of American Bacteriologists.¹

It is to be regretted, from a purely descriptive standpoint, that the organisms were not grown upon media prepared exactly according to the recommendations of the American Committee; but, since the main issue concerned the factors influencing toxin production, and since it was impossible to carry on two entirely separate sets of observations, a slight modification of these recommendations was employed for reasons which are given below.

Notwithstanding the use of these methods, a comparison of the biochemical peculiarities of the cultures chosen for this study reveals many points of difference between them—such as the production of a pellicle on bouillon by one, while another gives a diffuse cloudiness, the presence or absence of the cholera-red reaction, variations in the growth on potato, or in the type of the liquefaction of gelatin, etc. Many of these points of difference are

¹ *The Reports and Papers of the American Public Health Association*, 1898, XXIII, p. 60; or, for a brief summary of this report, *vide* L. Grimberty, on the Diagnosis of Bacteria by their Biochemical Functions, *Arch. d. Parasitologie*, 1903, VII, p. 304.

still emphasized in bacteriological literature, especially in the descriptions of single species. A careful preliminary study of one of these cholera cultures ("579") revealed such a wide variation in its morphology and in some of the details of its cultural characteristics, that I was forced to the conclusion that they could not be seriously considered in species description—since they are variations which will occur at intervals in the same culture.

It is hoped that this study, which was carried out under more uniform conditions than can be attained by older methods, will emphasize the variability of some bacteria and in a measure further the investigation of those factors entering into the production of such variations.

I have decided to present the subject-matter in the following order:

- I. A description of the method of preparing and neutralizing the media.
- II. The source, isolation, biochemical peculiarities, and variations of culture "579," with special reference to—
 - (a) The demonstration of the cholera-red reaction.
 - (b) The liquefaction of gelatin.
 - (c) The optimum reaction.
 - (d) The production of alkali.
- III. A description of the source and isolation of five other cholera cultures and of their resemblance to one another, and to culture "579."
- IV. Their growth in the presence of carbohydrates.
- V. Their relationship as shown by agglutinating and bactericidal sera.
- VI. Their pathogenicity.
- VII. Their morphology and pleomorphism.
- VIII. Summary and conclusions.

During this comparative study of a number of cultures from different sources, every precaution was taken to avoid contaminating one strain with portions from another culture, and the purity of each was controlled by frequent microscopical examinations and plating in gelatin or agar.

I. A DESCRIPTION OF THE METHOD OF PREPARING THE MEDIA.

One of the chief modifications of the methods recommended in the "Procedures" concerns the way in which the media was neutralized and the desired reaction obtained. The recommended method consists, briefly, in titrating a portion of the medium, as near the

boiling point as possible, with phenolphthalein as an indicator; obtaining an accurate neutral point by the addition of normal sodium hydroxide to the bulk of the medium, and then adding sufficient normal hydrochloric acid to give the desired reaction. In this work the acidity or alkalinity to phenolphthalein was adjusted by the addition of normal sodium hydroxide alone, and unless otherwise stated the reaction refers to that established before sterilization. The term "final reaction" indicates the reaction of the medium after sterilization or just before its use. The sign (+) stands for acidity, while (—) indicates alkalinity. The figures are in per cent. Thus + 1 means 1 per cent¹ acid with phenolphthalein as an indicator (slightly alkaline to litmus).

This modification was adopted for the following reasons: Our *préparateur* made some bouillon in which the cholera spirillum would not grow at 35°–37°, whereas *B. coli* did so in it, luxuriantly, at that temperature. Upon investigating this phenomenon it was found that if, after neutralization with sodium hydroxide, the precipitated acid albumins were first filtered and then the hydrochloric added, the acid may exert a germicidal or inhibiting effect upon the cholera spirillum—the degree of inhibition apparently depending upon the thoroughness with which the acid albumins have been removed. Further, this inhibiting effect is more marked at body than at room temperature.

I have not been able to reproduce this phenomenon at every trial. Whether this is due to a variation in the amount of acid albumins actually removed on neutralization (it is well known that a portion of the precipitate formed by neutralization redissolves in an excess of alkali), or to some undetermined cause, the fact remains that such a fluid will sometimes completely inhibit the growth of the cholera spirillum, but not that of *B. coli*. It seems as if work with chemically pure solutions might furnish another biological proof of the existence of ionproteid compounds and also an instance of a *specific* (?) *antitoxic action exerted by a proteid*. This would not be the only example of such an antitoxic action, for, as shown by Kahlenberg and True,² hydrogen and various metallic

¹One per cent acid or alkaline to phenolphthalein would, in chemical terms, represent 10 cubic centimeters of a normal acid or alkali to 1,000 cubic centimeters of the medium employed, or N/100.

²The Theory of Electrolytic Dissociation, H. C. Jones, 268–270. In my case, however, the action may possibly be due to free H ions from the hydrochloric acid, since it is a well-known fact that even small traces of acetic, hydrochloric, or citric acids completely destroy the growth of *Spr. cholerae*.

Kations exert a marked toxic action, at certain concentrations, on the germinating seedlings of *Lupinus albus*. The copper ion was found to be especially toxic and the seedlings barely lived in a solution containing a gram-molecular weight of copper ions in 51,200 liters of solution. But, when the copper ion was in combination with an organic complex, as in Fehling's solution, the roots would grow in a solution of this salt which contained a gram-atomic weight of copper in 400 liters.

The American committee does not recommend the removal of the acid albumins preliminary to the addition of the acid, but since Schultz-Schultzenstein¹ has shown that in fluids containing albumin or peptone or both, 0.097—0.217 per cent of HCL will destroy the cholera spirillum in an hour; and since further, as demonstrated by Smith,² hydrochloric acid is destructive to diphtheria toxin; and again, since Ritchie³ has pointed out a similar destructive action upon tetanus toxin, it was deemed advisable to leave the hydrogen ion out of the media.

The method of sterilization indicated in the "Procedures" was followed with the exception that bouillon and agar were sterilized in the autoclave at 120° for half an hour. This prolonged sterilization at a high temperature was found necessary and expedient on account of some very resistant spored organism encountered during the last hot season. It does not noticeably affect the nutritive qualities of agar or bouillon, although the initial acidity is raised about 0.5 per cent. When — 1 agar or bouillon is subjected to such an autoclaving a precipitate is usually thrown down. On filtration and further autoclaving the alkalinity is found to have been diminished by about 0.5 per cent.

This discrepancy, according to the "Procedures," "is perhaps due to side reactions which are not understood." The usual formation of a precipitate, which seems to vary in proportion to the amount of alkali added, with the simultaneous reduction in the alkalinity of the medium, seems to point to the hydrolysis and separation of insoluble compounds under the influence of the alkali.

¹ Schultz-Schultzenstein: Zur Kenntnis der Einwirkung des menschlichen Magensekrets auf Choleravibrionen. *Cent. Bakt. 1st Abt.*, 1901, XXX, 785-790.

²Theobald Smith: The Relation of Dextrose to the Production of Toxine in Bouillon Cultures of the Diphtheria Bacillus. *Jour. of Exper. Med.*, 1899, IV, 383.

³J. Ritchie: Artificial Modifications of Toxins with Special Reference to Immunity. *Journal of Hygiene*, 1901, I, 130.

Contrary to a statement made in the footnote on page 72 of the "Procedures," I have several times noted the evolution of ammonia when bouillon is boiled after the addition of the fixed alkali. As might be expected, this is most marked in the case of fermented bouillon, as illustrated in the following instance:

Three thousand cubic centimeters of sugar-free bouillon had an initial reaction of + 3.0; 2.5. per cent (75 cubic centimeters) normal sodium hydroxide was added and thoroughly mixed. This should have given a calculated reaction of + 0.5. The solution, containing a dense flocculent precipitate, was boiled for two minutes. During ebullition an appreciable quantity of ammonia was given off which could be detected both by the odor and by the blueing of red litmus paper moistened with distilled water. The solution was filtered, brought up to 3,000 cubic centimeters by the addition of distilled water, and then showed a reaction of + 1.0—i. e., the acidity had been raised 0.5 per cent over the calculated one.

A similar change occurs in unfermented meat extract, but here the escape of volatile alkali takes place more slowly.

II. THE SOURCE, ISOLATION, BIOCHEMICAL PECULIARITIES, AND VARIATIONS OF CULTURE "579."

NECROPSY No. 579.

Emeterio Darita, age 28 years, male, Filipino, residence, Lorchia *Horatio* (boat on the Pasig River). Died April 17, 1903, after an illness of ten hours. Clinical diagnosis, cholera. Autopsy performed three and three-fourths hours after death.

It was learned that the man had numerous stools and considerable vomiting before death.

The body was that of a very muscular man. There was not much shrivelling of the skin. The face was in repose. The feet were in extreme flexion. The fingers were in forced flexion and could not be straightened. The skin of the palms, the soles of the feet, the fingers, and the toes was shrivelled, the tissue beneath it being dry and comparatively bloodless. On the soles of the feet were a number of blister-like elevations, varying from about 2 to 20 millimeters in diameter. These were found to be perfectly dry on section. The white of the eyes was icteric. The muscles were very firm. On section the body tissues appeared abnormally dry. The superficial glands were not enlarged.

The *thoracic cavity* contained no fluid. There were a few rather firm, fibrinous adhesions between the lungs and the anterior thoracic walls. Posteriorly, the lower part of both lungs was bound down

by rather firm, fibrinous adhesions. The *thoracic organs* were covered by a scanty adhesive secretion.

The *lungs* were somewhat emphysematous throughout. The *pericardial cavity* contained a small amount of sticky secretion.

The *heart* was about normal in size and was in firm systole. It contained a considerable amount of thick, very dark, clotted blood. The blood which oozed from the larger arteries and coronary vessels was likewise of a very dark color and semicoagulated consistency. Otherwise the heart appeared normal. There was a persistent thymus gland about 5 by 15 millimeters in size.

The *peritoneal cavity* contained no fluid. The appendix was normal. The surface of the visceral and parietal peritoneum was covered with a scanty very adhesive secretion, which dried readily on exposure to the air. The parietal and visceral peritoneum throughout was of a rosy pink color.

The *spleen* was somewhat small in size and rather soft, and showed no particular changes on its surface or on section.

The *gall bladder* was filled with dark-green bile.

The *liver* was about normal size. The surface appeared somewhat cloudy, and on section the lobular markings were indistinct.

The *kidneys* were of about normal size; the capsules stripped readily, leaving a dark-red surface, on which the stellate veins stood out prominently. On section, the cortical and medullary markings were very indistinct.

The *stomach* contained a considerable amount of fluid substance, with many well-preserved rice granules. Its mucosa showed no particular change. The *intestinal tract* throughout contained a quantity of whitish, mucoid substance, which showed many white flocculi or rather flecks of a white albuminoid material. The secretion was very slippery while wet, but became very sticky on drying. The mucous surface of the duodenum and jejunum showed no change, but appeared rather whiter than normal. The mucosa of the upper two-thirds of the ileum showed no particular change, but a few of the solitary follicles were enlarged. In the lower third of the ileum the solitary follicles and Peyer's patches were quite generally enlarged, and some of the Peyer's patches appeared congested through the mucosa. The enlargement of the lymph follicles was most marked just above the ileo-cecal valve, where they were about 2 millimeters in diameter. In several places in the lower

portion of the ileum the mucosa was almost completely desquamated for a distance of several inches. The *pancreas* appeared rather pale on section.

The *mesenteric glands* were enlarged throughout to the size of almonds, and were pale on section (a common post-mortem finding here).

The *colon* and the upper portion of the *rectum* contained fluid contents similar to that described above. Here the mucous surface showed no particular changes, except that the solitary glands were enlarged—especially in its upper portion.

Anatomic Diagnosis.—Cholera; acute follicular and necrotic enteritis; follicular colitis; acute parenchymatous nephritis; acute parenchymatous hepatitis; adhesive pleuritis; emphysema of the lungs; persistent thymus.

Tissues from the organs hardened in Zenker's solution.

Smears.—Ileum (carbol fuchsin 1:10): Showed almost pure culture of slender rods, often curved, quite numerous; many degenerated, columnar epithelial cells. (See fig. 2.) Spleen (Gram and Safranin): No bacteria. Heart's blood (Gram and Safranin): No bacteria.

Method of isolation.—Cultures were made from the ileum according to the Schottelius enriching method. Peptone solutions (1 per cent Witte's peptone and 0.5 per cent NaCl in distilled water) with a reaction of +1, +0.5, neutral, and —1 were inoculated and kept at 35°–37°.

In twenty hours the +1 tube showed a dense layer of growth near the surface, with beginning pellicle formation. A hanging drop preparation from the surface showed short, actively motile curved rods—apparently in pure culture.

The +0.5 and neutral tubes were well clouded, no pellicles.

The —1 tube was faintly clouded with a thin pellicle in the process of formation. It showed actively motile curved rods in the hanging drop.

The addition of ten drops of chemically pure sulphuric acid to each of the tubes gave a distinct indol reaction, which was most marked in the +1 tube.

Twenty per cent gelatin plates inoculated from the surface growth of the +1 peptone tube were kept at 18°–28°. In twenty hours pinhead-sized areas of liquefaction were produced. These were cir-

cular, well defined, and contained motile masses of growth of a broken-up, refractile character.

A pure culture was obtained on + 1 agar.

Biochemical peculiarities and variations.—On + 1 agar a luxuriant dirty-white growth is seen in twenty-four hours at 35°–37°. Later the edges become crenated—especially if the agar is somewhat dry. The condensation water is densely clouded. In old cultures spine-like processes may project from the edges of the growth, which is much more luxuriant on fresh, moist agar than on the same medium with a slightly dry surface. (For the optimum reaction see under “Liquefaction of gelatin.”)

In + 1 bouillon the growth is somewhat variable. On one occasion the bouillon may be uniformly clouded; in the following, this is termed the “anaërobic type of growth.” Again it may be clouded with a dense layer of growth near the surface, which soon forms a pellicle. Below this is termed the “aërobic type of growth.” In a stained preparation from a forty-eight hour culture the organisms are thicker and more curved than from one in peptone solution grown under like conditions. The morphology varies with the reaction of the medium. The character of the growth is independent of the presence or absence of muscle sugar, and is apparently due to the predominance of the aërobic type of the organism on the one hand, or of the anaërobic type on the other. I came to term these aërobic and anaërobic types of growth as a matter of convenience, but a better theoretical explanation is furnished by assuming that the difference is due to a variation in the specific gravity of the bacterial cells. If a number of bouillon tubes be inoculated from an agar slant, and kept under like conditions, some will show the aërobic type while others will be uniformly clouded and may remain so or form a pellicle at a later date. The type of growth can be transmitted by further inoculations in bouillon, although, in the case of the anaërobic type, there is a tendency toward a cropping out of the aërobic one.

The production of a pellicle in fluid cultures.—As with the diphtheria bacillus, and other organisms, the habit of producing a pellicle in fluid cultures can be firmly established by transferring a portion thereof through a series of fluid cultures. “579 A” is one, which after being transplanted in this manner at intervals of three or four days for a couple of months, shows little or no ten-

dency to grow in the deeper parts of the bouillon, while a dense layer appears near the surface of the fluid and a pellicle is formed in much less time than when the training process was initiated, and the same result can be obtained much more rapidly by using the pellicle formed on a liquefied gelatin culture. The whole process is, in fact, one of artificial selection. Inoculations from such cultures are usually made from the upper layers of the fluid and hence a series of such inoculations yields an artificially selected race of organisms of low specific gravity.

So far as indol and alkali productions are concerned, there is no difference in the action of the aërobic and anaërobic type of organisms. (See alkali production.)

It is evident that the presence or absence of a pellicle in bouillon cultures is of little value in the differentiation of species.

Litmus milk is acidified and coagulated in forty-eight hours. (Control tubes remain sterile.) In about four days a firm clot is formed with separation of the whey and partial reduction of the litmus. The fermentation of lactose bouillon is evidence that this culture produces lactase.

+ 1 *glucose bouillon* is faintly clouded, but the growth occurs mostly at the bottom of the test tube as a stringy, viscous mass. There is no apparent increase after twenty-four hours. (See growth in the fermentation tube.)

On potato (unneutralized) the growth is variable, sometimes none appearing, or again a slight dirty yellowish one may be seen in three or four days. This variability is probably due to a difference in the acidity of the potatoes used.

Solidified ox serum is rapidly digested.

When grown anaërobically (pyrogallic acid method), in + 1 *glucose agar*, growth appears along the line of inoculation, but the culture is no longer viable after the second anaërobic transplanting.

(a) ON THE DEMONSTRATION OF THE CHOLERA-RED REACTION.

Immediately after isolation from the body, this organism gave a pronounced cholera-red reaction upon the addition of ten drops of chemically-pure sulphuric acid to cultures grown in peptone solution during eighteen to twenty hours at 35°–37°. Since then, this reaction has only appeared at intervals—even in solutions

prepared from Witte's peptone,¹ which had been set aside as "Proper for Indol."

All the cultures mentioned in this article have shown the same variation from time to time.

For some time peptone solutions of various reactions were used both in isolating the cholera spirillum at autopsy, from stools, water, etc., and in testing for cholera red, but without any constant results which might determine whether any one reaction favors surface growth or the demonstration of the reaction. Dunham's peptone solution containing 1 per cent Witte's peptone and 0.5 per cent sodium chloride in distilled water has a final reaction of $+0.5$, and has given the best results on the whole. This solution has such poor nutritive qualities for many species of intestinal bacteria that it is especially suitable for isolation by the Schottelius enriching method.

Upon investigating this uncertainty of the cholera-red reaction, I determined to try sugar-free bouillon, which has been shown by Smith² to be such an excellent culture fluid for the production of indol by bacteria. In the first batch of this medium, these cultures gave excellent cholera-red reactions, but in two subsequent ones the reaction failed to appear. These three media were shown to be free from nitrites and fermentable sugars, by testing with *B. coli*, as recommended by Smith. In addition to sugar-free bouillon, four different peptone solutions were tested, namely: *Peptone Sicca cum Sale*, R. Nishiyama, Osaka, Japan; *Peptone Carne*, E. Merk, Darmstadt; and two samples of *Peptonum Siccum*, Friedr. Witte, Rostock—one of which had been marked "Proper for Indol." These too were found to be free from fermentable sugars and nitrites, but all failed to yield cholera red. However, they gave the indol reaction upon the addition of a trace of sodium nitrite.

As is well known, the demonstration of the cholera-red reaction depends upon the fact that an organism not only forms indol, but

¹ It may be noted that this so-called "peptone" consists of a mixture of albumoses and contains only a minimal quantity of true peptone—Torald Sollmann, Witte's Peptone: Its Dissociation, and its Combination with Acid and Alkali. *Amer. Jour. of Physiol.*, 1902, VII, 203; on the other hand, there are "peptones" on the market, such as that manufactured by the firm of Chapoteau, which contain as much as 50 per cent of pure peptone (J. P. Pawlow: *The Work of the Digestive Glands*, 1902, 96).

² Theobald Smith: A Modification of the Method for Determining the Production of Indol by Bacteria. *Jour. of Exper. Med.*, 1897, II, 543-547.

also either produces nitrites or reduces nitrates to nitrites. Having a premonition that the inconsistency of the reaction might depend upon a variation in the amount of nitrates present in different lots of peptone or meat extracts, or upon their accidental introduction on one occasion and not on another (when Cross and Blackwell's table salt is used, much more constant results are obtained, than when C. P. sodium chloride is employed), I prepared peptone solutions in the manner above indicated, but in addition to the C. P. sodium chloride, I introduced 0.01 per cent C. P. sodium nitrate (1 cubic centimeter of a 10 per cent solution per liter). In such a solution the cholera-red reaction is not only constant, but it appears more promptly and is more intense than usual. Control peptone solutions, not containing sodium nitrate, failed to give the reaction. (All the cholera cultures mentioned in this article give the reaction constantly and promptly in this medium.)

Since completing this work, I have found that Max Bleisch,¹ as long ago as 1893, emphasized the necessity of introducing nitrates into the peptone solution.

There seems to be some evidence that the nitrate content of meat extract or "peptone" may vary; and this may account for some of the discrepancies in species description, for an organism attributed with nitrifying powers may only possess the ability to reduce nitrates to nitrites—a property common to many species of bacteria.

Thus, to cite an instance: Last year Woolley and Jobling,² working in this laboratory, described cultures of *B. bovissepticus* which gave a distinct cholera-red reaction upon the addition of chemically-pure sulphuric acid to cultures grown for from twenty-four to thirty-six hours in Dunham's peptone solution. At that time the cholera cultures here mentioned also gave the same reaction. I have recently tested two of these cultures of *B. bovissepticus*, and find that they fail to give the cholera-red reaction in the peptone solution in which the cholera cultures fail to do so, but yield it promptly when grown in the peptone solution containing 0.01 per cent sodium nitrate.

As shown by Kastle and Elvone,³ hyponitrous acid, nitrous acid, nitrites,

¹ Max Bleisch: Ueber einige Fehlerquellen bei Anstellung der Cholerarothreaktion und ihre Vermeidung, *Zeit. für Hyg. und Infekt.*, 1893, XIV, 103-115.

² P. G. Woolley and J. W. Jobling: A report on Hemorrhagic Septicemia in Animals in the Philippine Islands, *Bull. No. 9, Biological Laboratory, Bureau of Government Laboratories*, p. 8.

³ Kastle and Elvone: Oxidation and Reduction in the Animal Organism and the Toxic Action of Powerful Oxidizing and Reducing Substances. *Amer. Chem. Jour.*, 1904, 31, 195-207.

etc., are, in part, converted into nitrates in the animal body. And as has been demonstrated by Mayo,¹ when potassium nitrate is fed to cattle, a chemical test for nitrates can sometimes be obtained after their death, although, as a rule, nitrates are rapidly reduced to nitrites in the tissue fluids.

It seems probable that the use of Smith's sugar-free bouillon, containing 0.01 per cent sodium nitrate, would furnish a means of testing the production of indol and the simultaneous reduction of nitrates by many bacteria.

(b) ON THE LIQUEFACTION OF GELATIN.

At the time of isolation, this organism showed active proteolytic properties—liquefaction of 20 per cent gelatin appearing within twenty-four hours, at 18° to 28°, and rapidly spreading to the sides of the test tube as a shallow, circular, pan-shaped area. Then the liquefaction descended progressively from above downwards, involving the whole width of the tube, with a slight funned-shaped depression in the center along the needle puncture. Careful data concerning the reaction and dryness of the gelatin were not kept at the time, but some variations in the rate and character of liquefaction were noticed. Further work at the time being impossible, the original agar culture was kept in the ice chest (transplants on +1 agar being made at intervals of every two months) for eight months. The organism still showed the above type of liquefaction (which is often described as being characteristic of *Spirillum Finkler Prior*) at 18°–28° in 20 per cent gelatin, which had a final reaction of +1.2. At the same temperature, in fresh 20-per cent gelatin, which had a final reaction of +2, the organism slowly produced, in the course of three days, a small turnip-shaped area of liquefaction which, drying at the surface, left a small bubble-like depression—that is, it produced the type of liquefaction which was described by Koch as being characteristic of the cholera spirillum.

In two separate trials with the same gelatin (+2) at 35°–37°, the inoculated material precipitated, and no growth or liquefaction occurred.

In +1.5 gelatin at 10°–15° no growth occurred in ten days, but rapid liquefaction took place on change to 18°–25°.

When grown anaerobically (pyrogallie acid method) at 18°–28°

¹N. S. Mayo: Cattle Poisoning by Nitrate of Potash, *Bull. No. 49* (1895), Kansas State Agricultural College.

in +1 gelatin containing muscle sugar, growth appears along the stab but no liquefaction takes place in three days.

Before detailing some experiments performed to determine the factors influencing variation in the type of liquefaction, it may be well to note some of the points brought out in the literature on this subject.

The proteolytic ferments of bacteria are only active in a medium alkaline to litmus, and it takes but a small amount of acid to hinder their action. This is in accord with the behavior of trypsin. When carbohydrates which can be so fermented as to form acids are present in gelatin, its liquefaction is inhibited. In 1898 Auerbach,¹ working with a number of liquefying bacteria, showed that the inhibiting power of glucose exceeded that of lactose, and that in the case of *B. vulgare* the acid products of fermentation inhibited the formation of the ferment itself. It seems that for the production of the ferment a medium containing albumin and the access of free oxygen is necessary. According to Liborius² liquefaction of gelatin takes place very slowly in the absence of oxygen—with the exception of the case of some anaërobes.

According to T. Sollman (loc. cit, p. 211), "Kühne investigated the action of *B. subtilis* and *B. prodigiosus* on solutions of protalbumose from the chemical standpoint, and found that the phenomena resemble closely those of tryptic digestion. The conversion to tyrosin, leucin, and tryptophan was often almost complete." Again, according to Gotschlich,³ "Kalischer in experiments to determine how much of the casein splitting was due to the ferment and how much to the living cells, found that the ferment was able to produce peptone, leucin, tyrosin, as well as ammonia and aromatic oxyacids—in which its action also is in harmony with that of trypsin."

The melting point of gelatin undoubtedly plays a part in influencing the type of liquefaction which will occur at any given temperature. In my own experience the addition of alkali lowers the melting point. Thus, neutral gelatin which will not congeal at 18°–28° will do so in the ice chest, and +1 gelatin is not as solid as +1.5 gelatin at the same temperature. An interesting communication by Paul von Schroeder⁴ throws some light on this subject. "When a gelatin solution is heated at 100°, and samples are taken out at intervals and placed in a thermostat at 25°,

¹Auerbach: Ueber die Ursache der Hammung der Gelatinverflüssigung durch Bakterien durch Zuckerzusatz. *Ref. C. B.*, II Abt. 1898, IV, 492–494.

²Liborius: Beiträge Zur Kenntniss des Sauerstoffbedürfnisses der Bakterien, *Ztschr. f. Hyg.*, 1886, I, 115–176.

³E. Gotschlich: Handbuch der Pathogenen Mikroorganismen, Kolle u. Wassermann, 1903, I, 107.

⁴Paul von Schroeder: Phenomena of the Setting and Swelling of Gelatin, Review: *Jour. of the Chem. Soc'y*, 1903, vol. 84, ii, 721.

their viscosity being determined five minutes later, it is found that the values of the viscosity diminish, as the duration of the heating at 100° increases, ultimately becoming constant.

"This change is attributed to a process of hydrolysis * * *.

"Certain salts increase the viscosity, magnesium salts exerting the greatest influence * * *.

"The effect of hydrochloric acid and sodium hydroxide on the behavior of gelatin solutions was similarly studied. The process of hydrolysis is accelerated by both hydrogen and hydroxyl ions, and the final value of the viscosity thus attained after hydrolysis is lower than that reached in pure or salt containing gelatin solutions."

Again, according to Rousseau,¹ if gelatin be dialyzed, so as to remove the calcium salts contained therein, one obtains a solution which, sterilized in an autoclave at 120° for twenty to thirty minutes, solidifies upon cooling.

In order to determine what influence the reaction and dryness of the gelatin exert upon the type of liquefaction produced by a given cholera culture, the following experiment was performed: Nutrient gelatin was prepared containing 20 per cent gold label gelatin, 1 per cent Witte's peptone and 0.3 per cent Liebig's beef extract. It was divided into halves and to each portion normal NaOH was added, one-half receiving more than the other. After sterilization one portion showed a final reaction of +0.8, while that of the other was +1.0. In addition to this, another sample of gelatin, slightly darker in color but prepared in the same way, which had been kept on ice for three weeks and showed some evaporation and a final reaction of +1.5, was used. This one was melted and resolidified before inoculation. Each sample contained a small amount of muscle sugar as shown by subsequent fermentation with *B. coli*.

Four tubes from each of these samples were then inoculated from a twenty-four-hour culture of "579" on +1 agar, which had been kept on agar transplants at 35°-37° for two and a half months. In forty-eight hours at 18°-28° there was quite a noticeable variation in the amount of liquefaction produced in the different sets of tubes. The amount of this, in the four tubes of any one of the three sets, was not exactly uniform, probably on account of a variation in the number of bacteria introduced at the time of inoculation,

¹Em. Rousseau: Influence of the Salts of Calcium upon the Solidification of Gelatin Sterilized at 120°, *Bull. Inst. Pasteur*, 1903, I, 719.

but the difference between the three sets was very noticeable. Any one of the four $+0.8$ tubes showed more advanced liquefaction than any of the $+1$ tubes, and the difference between the $+1$ and $+1.5$ tubes was still more marked, as shown in fig. 1.

It is often stated that "bacterial proteolytic enzymes, like trypsin, show increased activity in the presence of certain chemicals, such as sodium carbonate and salicylate."

So far as the action of certain ions upon the tryptic digestion of fibrin is concerned, A. Kanitz,¹ in reviewing the work of Dietz and confirming the quantitative experiments of Shields,² has shown that the optimum concentration of the hydroxyls from barium, strontium, and calcium hydroxides varies between $\frac{1}{70}$ and $\frac{1}{130}$ of the gram-molecule per liter. Determination of the electric conductivity and other physical constants shows that these alkaline earths are strongly and almost equally dissociated at these dilutions, and since the three hydroxides work at the same concentration he concludes that the kation is without influence and that the anion is alone active. He then calculated, from the per cent of hydrolysis of potassium carbonate in given dilutions, the concentration at which the carbonate of potassium exerted the most active influence on tryptic digestion and found this to be about $\frac{1}{200}$ of the gram-molecule per liter. He was unable to say that there was any difference in the mode of action of carbonate of potassium and the hydroxides of the alkaline earths. Kanitz concludes that the optimum for tryptic digestion is a liquid containing $\frac{1}{70}$ to $\frac{1}{200}$ of the hydroxyl ion ($\text{OH}=17$ gms.) per liter.

In order to test the above statement from a bacteriological standpoint, an experiment was performed as follows: I prepared one liter of nutrient gelatin by adding 20 per cent Gold Label gelatin, 1 per cent Witte's peptone, and 0.5 per cent sodium chloride to 1,000 cubic centimeters of distilled water; the ingredients were then dissolved by boiling; distilled water added to 1,000 cubic centimeters; the mixture then was divided into two parts of exactly 500 cubic centimeters each; each half titrated to phenolphthalein, and sufficient normal NaOH added to one-half to give a reaction of $+1$,

¹A. Kanitz: Ueber den Einfluss der Hydroxylionen auf die tryptische Verdauung. *Zeit. für physiol. Chemie.*, 1902, 37, 75-80.

²John Shields: Ueber Hydrolyse in wässrigen Salzlösungen. *Zeit. f. physikalische Chemie.*, 1893, 12, 167.

and an equal volume of normal Na_2CO_3 was then added to the other half; after cooling to 40° the whites of three eggs were added to each half; each portion was then boiled for three minutes, filtered through cotton, distributed and sterilized in the Arnold for twenty minutes on each of three successive days. The final reaction of the NaOH gelatin was $+1.7$, while that of the Na_2CO_3 gelatin was $+1.8$.

Six tubes of each were then inoculated from the same place on the edge of the growth of a twenty-four-hour agar culture of "579." and kept at 18° – 28° . Liquefaction commenced and progressed slowly but equally during several days' observation.

The results of Kanitz would not appear to give conclusive proof of his view that the Kation exerts no influence on the optimum reaction, as a glance at the table given in his paper will show, the variations between the three alkaline earths being quite marked at different temperatures. In Dr. Wherry's work he compared equivalent amounts of sodium hydroxide and sodium carbonate, and thus, while he had the same concentration of hydroxyl ions, he had, in the case of the latter reagent, twenty-five times the number of sodions present in unit volume. This latter fact would tend to show that the kation is without marked influence; at least in the case of sodions. However, these results show that the question is one which is barely touched and is well worthy of complete investigation by a use of the methods of physical chemistry in biology.—FREER.

An attempt was made to increase the proteolytic activity of this culture by transferring it at intervals of every few days from one gelatin tube to another. At the end of four months this culture showed no greater activity than another transplant of the same culture which had been kept on agar slants for the same length of time.

What has already been said concerning the influence of the reaction of the medium upon the rapidity with which the cholera spirillum is able to liquefy gelatin has a direct bearing upon the type of liquified areas it will produce in or upon gelatin plates. Further, when, as has been noted by many observers, the same culture gives rise to two distinct types of liquified areas, the difference may be explained by the relation of the plated organisms to their oxygen supply. Thus, an organism situated at the surface on account of its greater supply of free oxygen might be expected to produce a more rapidly spreading area of liquefaction than one more deeply situated, where the supply is *relatively* less. Moreover,

the colony at the surface would be of the shallow, turbid type with a greater area than that of the deeper colony where the organisms encounter a greater resistance of the surrounding gelatin, and in consequence of which they would be massed together—producing the refractile “ground-glass” type of colony. It has been noted that such “ground-glass” colonies, upon further growth, invariably break up into liquid areas with turbid contents and such breaking up occurs *pari passu* with a lessening of the surrounding resistance and an increase in the supply of free oxygen. That such a supply of free oxygen does exist in a thin layer of gelatin can be proven by covering the opening of a gelatin stab culture with a few drops of liquid gelatin. Here no liquefaction occurs until the organisms have spread nearly to the surface.

(c) ON THE OPTIMUM REACTION.

All the cultures mentioned in this article show much more luxuriant growth in eighteen to twenty hours, at 35°–37°, on fresh -0.5 than on fresh $+0.5$ agar. Furthermore, the maximum amount of growth is obtained on -0.5 agar in eighteen to twenty hours, while that on $+0.5$ agar does not equal it in thirty-six to forty-eight hours at the same temperature. The -0.5 agar was prepared from Liebig’s beef extract and had an initial acidity of 1 per cent acid to phenolphthalein. It was neutralized and brought to a reaction of -1 . After sterilization, the reaction was reduced to -0.5 . Since 20 cubic centimeters of normal NaOH were added in the first place, and part of this was lost in the precipitate thrown down by autoclaving, it contained between $1/50$ and $1/100$ of a gram-molecule per liter. This would seem to support the idea that the optimum conditions for the growth and multiplication of the cholera spirillum are such as will favor its proteolytic activity.

(d) ON THE PRODUCTION OF ALKALI.

Fifty cubic centimeters of Smith’s sugar-free bouillon¹ was placed in each of five 600-cubic centimeter Ehrlenmeyer flasks and autoclaved at 120°. Final reaction, $+1.5$.

Each was then inoculated with one loop from an eighteen-hour

¹For the method of preparation see *Jour. of Exper. Med.*, 1899, IV, 375.

evenly clouded sugar-free bouillon culture of "579," and the following table illustrates the rate of alkali production:

No. of flask.	Temperature.	Age of culture.	Reaction to phenolphthalein.	Remarks.
	°	Hours.		
1	30-35	24	+0.8	Dense cloudiness; no pel- licle.
2	30-35	48	+1.0	Dense cloudiness; no pel- licle; actively motile curved rods.
3	30-35	72	+0.2	Do.
4	30-35	96	Neutral.	Do.
5	30-35	120	-0.8	Dense cloudiness; no pel- licle; rods not so actively motile; few curved fila- ments.

Alkali production progresses equally as well when a pellicle is formed. It also occurred in unneutralized sugar-free bouillon with an initial acidity of +2.3. If the sodium chloride usually added to sugar-free bouillon be left out, no formation of alkali can be detected by titration with phenolphthalein—at least during five days. In control flasks of the same bouillon, plus sodium chloride, alkali production occurred about as rapidly as shown in the above table. This would seem to indicate that the alkali is produced by a conversion of NaCl into NaOH or Na_2CO_3 , and that the greater part of the alkalinity is owing to the formation of such substances rather than to ammonia, amine, and ammonium bases, to which it is usually attributed. However, it is also possible that sodium chloride exerts a catalytic (accelerating) action on the formation of ammonia.

III. A DESCRIPTION OF THE SOURCE AND ISOLATION OF FIVE OTHER CHOLERA CULTURES, AND OF THEIR RESEMBLANCE TO ONE ANOTHER AND TO CULTURE "579."

Cholera "Scout" is a culture obtained by the Schottelius enriching method from a stool sent to the laboratory from Caloocan on April 16, 1903. The patient was a native scout who died on the next day with typical symptoms of Asiatic cholera, which was epidemic in Caloocan and the surrounding country at the time. In its cultural characteristics it is indistinguishable from "579," excepting that litmus milk is acidified in twenty-four hours at 37°C , but no coagulation occurs in five days. Hence, like "579," it

produces lactase, and differs from it in the absence of the production of rennin.

Cholera "561" is a culture obtained by the Schottelius enriching method from Eugenia Holandes, a Filipina, 33 years old, who died on March 26, 1903, after an illness of three days. Autopsy, seven hours after death. To briefly summarize the post-mortem findings: The skin of the hands and feet is shrivelled; the feet are in extreme flexion with the toes in extension; the pleural and peritoneal membranes covered with a sticky secretion. Cloudy swelling of the solid organs. Ileum congested, especially in its lower half, the mucosa showing some patches of epithelial desquamation. Contents of ileum greenish black and containing much mucus. Old and advanced amebic colitis. Culturally it is indistinguishable from *Cholera "Scout."*

Cholera "A" is a culture obtained at autopsy by Dr. R. P. Strong some time in the fall of 1903, cholera being endemic in Manila at the time. Culturally it is indistinguishable from cholera "*Scout.*"

Cholera "City Moat" is a culture obtained by Mr. Lindquist, of the First Reserve Hospital laboratory, from the city moat near the hospital, about July, 1903. Cholera was endemic in Manila at the time. Culturally it is indistinguishable from cholera "*Scout.*"

Cholera "Pfeiffer" is a culture of that name brought by Dr. R. P. Strong from Germany. It has been grown on artificial media for a period of nine years, and during the past year has not been passed through animals. Culturally it is indistinguishable from cholera "*Scout,*" but it is very much more sensitive to the action of agglutinating sera.

"554-B" is a culture obtained on March 20, 1903, from a cholera autopsy. Morphologically it appears as short, curved, actively motile rods. It closely resembles the above cultures but does not agglutinate with the serum of a rabbit immunized against "579" nor with the serum of a cholera convalescent.

IV. THEIR GROWTH IN THE FERMENTATION TUBE IN THE PRESENCE OF CARBOHYDRATES.

Medium: Smith's sugar-free bouillon, which had a final reaction of ± 1.5 containing 1 per cent of glucose, maltose, saccharose, and lactose. One per cent of starch was added to some of the same bouillon and autoclaved after distribution; the initial reaction was

not changed. When inoculated with "579" and kept at 35°-37° the following results were noted:

Bouillon.	Gas.	Reaction of contents of bulb and neck on fourth day.	Gas in control tubes inoculated with <i>B. coli</i> (fourth day).	Remarks.
Glucose.....	0	+3.3	30 per cent; $\frac{H}{CO_2} = \frac{2.5}{1}$	Maximum growth attained in twenty-four hours. Bulb and closed branch turbid; no pellicle. Agar slants inoculated after twenty-four hours from the bulb or neck remain sterile.
Maltose.....	0	+3.2	45 per cent; $\frac{H}{CO_2} = \frac{3}{1}$	Maximum growth attained in twenty-four hours. Bulb and closed branch turbid; no pellicle. Agar slants inoculated on the fourth day from the bulb or neck remain sterile.
Saccharose.....	0	+3.5	No gas; closed arm cloudy.	Do.
Lactose.....	0	+4.0	40 per cent; $\frac{H}{CO_2} = \frac{2}{1}$	Maximum growth attained in twenty-four hours. Bulb and neck turbid. Closed branch clear; no pellicle. Growth more dense than in other sugars. Agar slants inoculated on the fourth day from bulb or neck show a luxuriant growth in twenty-four hours at 37°; actively motile curved rods in the hanging drop.
Starch ¹	0	+4.0	No gas; closed arm cloudy.	Maximum growth attained after twenty-four hours. Culture viable on fourth day as per lactose tube.

¹A test tube containing the same starch solution became densely turbid and a well-marked pellicle was formed. On the fourth day the acidity had reached 3 per cent. The closed arm of a fermentation tube was filled with this culture and -1 bouillon added. When inoculated with *B. coli* 30 per cent of gas was formed in forty-eight hours $\frac{H}{CO_2} = \frac{3}{1}$.

It will be noticed that in glucose, maltose, and saccharose bouillon there was growth in the closed arm as well as in the bulb, and that the acids produced were of such a character as to destroy the vitality

of the organism. On the other hand, in the case of the lactose and starch bouillon, no growth occurred in the closed arm, and, although a greater quantity of acid was produced, the organism was still viable on the fourth day.

In another series of experiments, in which 0.5–1 per cent glucose bouillon (final reaction = +1.5) was distributed in small flasks and inoculated from the same culture and kept at 35°–37°, the maximum amount of acid (3–3.5 per cent) was produced in twenty-four hours and transplants made at that time remained sterile.¹

Again, enough normal NaOH was added to sugar-free bouillon to give a calculated neutral reaction. The final reaction after autoclaving was +0.7. A sterile solution of glucose, amounting to $\frac{1}{16}$ per cent, was then added and the flask inoculated and kept at 28°. In four days the acidity had been raised 0.5 per cent and the culture was still viable. The experiment was not carried on for a sufficient length of time to note whether the acid produced would be finally neutralized by such alkali production as normally takes place in sugar-free bouillon, but this is hardly probable as the growth, in the presence of even such a small per cent of glucose, is rapidly precipitated and forms a very viscous sediment.

The other cultures grown in solutions of these carbohydrates (reaction +1–+1.5) yielded similar results as shown in the following table:

Bouillon.	"Scout."	"561."	"A."	"City moat."	"Pfeiffer."	Remarks.
Glucose.....	+3.8	+3.6	+3.8	+3.5	+3.5	Titration on fourth day. Character of growth and fate of culture as per culture "579."
Maltose	+4.0	+4.0	+3.0	+4.0	+4.4	
Saccharose	+3.0	+3.0	+3.0	+3.0	+3.0	
Lactose	+3.8	+4.0	+4.5	+4.3	+4.0	
Starch	+2.8	+3.0	+3.0	+2.3	+2.8	

Buxton,² in an excellent discussion on bacterial enzymes states that "cholera then produces amylase, maltase, but no invertase,

¹ See analogy in the case of the diphtheria bacillus (Th. Smith, loc. cit., p. 382). It is extremely probable that any toxin formed by the cholera spirillum would be destroyed in a manner similar to that which takes place in diphtheria cultures.

² Buxton: Mycotic Enzymes. *Am. Med.*, July 25, 1903, 138.

lactase, nor inulase." These cultures seem to produce both lactase and invertase. The sugars used were prepared by Merk.

E. Gotschlich (loc. cit., p. 106) states that Fermi and Montesano found that invertin occurred inconstantly in the cholera spirillum and spirillum of Metchnikoff.

V. THEIR RELATIONSHIP, AS SHOWN BY AGGLUTINATING AND BACTERICIDAL SERA.

In applying the Gruber-Durham test to the study of the identity or relationship of the following cultures, a number of facts observed by others influenced both the choice of the method employed and the interpretation of the results.

In making a quantitative determination of the power of a given serum to produce a complete agglutinate when tested on a series of cultures, probably no one factor will influence the production of discordant results so much as quantitative variations between the agglutinin and the agglutinable substance. Thus, to cite an instance, an emulsion of culture "579" in 0.8 per cent sodium chloride solution was tested against the serum of a cholera victim diluted 1:100; agglutination was partial in thirty minutes and not complete until sixty minutes at 28°. The same emulsion was diluted with an equal quantity of the salt solution and then at 1:100 gave a complete reaction in thirty minutes at the same temperature.

A dense suspension of a culture when mixed with a powerful serum at a low dilution may give a prompt but only a partial reaction—numerous bacilli remaining unaffected in the serum which is now freed from agglutinins by the precipitated bacteria. On the other hand, as the dilution of the serum is increased, a similar disproportion is produced with the same result.

On account of variations in the density of the growth in bouillon, which the cultures studied at times show, emulsions of the bacilli in 0.8 per cent sodium chloride solution were exclusively employed. The cultures were grown on +1 agar for eighteen to twenty hours at 35°–37°, and the emulsions made to correspond as nearly as possible with the density of a twenty-four-hour typhoid culture according to the method employed by Smith¹ in the comparative

¹Theobald Smith: *Jour. of Exper. Med.*, 1898, III, p. 465.

study of tubercle bacilli. They were allowed to stand for ten minutes in order to give time for the coarser particles to settle. Such an emulsion is microscopically free from clumps, and the rods retain their active motility in the control drops for an hour or more.

The serum was diluted with 0.8 per cent sodium chloride solution in Thoma-Zeiss blood pipettes, and a loopful of this serum was then mixed with an equal quantity of the emulsion and examined from time to time with the 1/6 objective. It will be noted that the dilution of the serum in the drop was always twice that in the diluting pipette. Control hanging drops of the emulsion were always made and examined before and at the close of each experiment. The microscopic method was employed because it was believed that the end of the reaction can be more accurately determined and any differences in the character of the clumps noted.

It is a well-known fact that organisms, which have been grown for a long time upon artificial media, are more sensitive to the action of homologous sera than they are when their pathogenicity has been raised. Typhoid cultures recently isolated from the body sometimes show a marked resistance to agglutination with the patient's serum as compared with old laboratory cultures. As shown by F. Hamburger¹ the agglutinability of cholera cultures diminishes with an increase in virulence.

AGGLUTINATION WITH THE SERUM OF A CHOLERA VICTIM.

The history of the serum is briefly as follows: Candido Nugin, a Filipino, 19 years old, died at the San Lazaro Cholera Hospital on January 8, 1904. He was ill for thirteen days; had rice-water stools during the acute stage of the disease, passed into the typhoid stage, and died on the thirteenth day with symptoms of acute nephritis. At the autopsy six hours after death, the kidneys showed acute parenchymatous nephritis; there was cloudy swelling of the liver and heart muscle. The ileum was still in a congested state, but its mucosa was in fairly good condition. Smears from the ileum showed a number of thin curved rods, mixed with many other organisms. No cultures were made.

The following table shows the agglutinating action of the serum from the heart's blood of this patient in such dilutions as were tested.

¹F. Hamburger: *Wien. Klin. Woch.*, 1903, XVI, 97-98.

An accident to the serum prevented the determination of its agglutinating limit:

Culture.	Temperature.	Dilution of serum.	Result.
	0		
"579"	28	1:10	Small motile clumps in seventeen minutes; complete in thirty minutes.
"579"	28	1:10	Small motile clumps in ten minutes; complete in forty minutes.
"Scout"	28	1:10	Almost complete in thirty minutes; complete in sixty minutes.
"561"	28	1:10	Do.
"A"	28	1:10	Almost complete in thirty minutes; not complete in sixty minutes.
"City moat"	28	1:10	Complete in thirty minutes.
"Pfeiffer"	28	1:10	Complete in twenty minutes.
"554b"	28	1:10	Negative during an hour's observation.

My own serum diluted 1:20 produced no agglutination during forty-five minutes' observation.

In this experiment no attempt was made to use salt-solution suspensions of equal density and the variation in the time when complete agglutination occurred is noticeable.

AGGLUTINATION WITH IMMUNE RABBIT SERUM.

A rabbit was injected with 0.8 per cent sodium chloride suspensions of culture "579" grown on +1 agar for twenty-four hours at 35°-37°. It received the contents of about six agar slants subcutaneously and intraperitoneally during two months. In this twenty-four-hour-old serum the agglutinating limit is not great, but is considered sufficient for the following comparative and quantitative estimations:

Culture.	Temperature.	Dilution of serum.	Result.	Remarks.
"579"	25	1:100	+	Complete in thirty-five minutes; small, compact clumps. ¹
"579"	25	2:1000	-	Partial in twenty-five minutes; not complete in sixty minutes. ¹
"Scout"	28	1:100	+	Complete in thirty-five minutes; small, loose clumps. ¹
"561"	28	1:100	+	Do. ¹

¹The hanging drop was not examined during the five minutes previous to the given time, hence it is probable that the table indicates a greater uniformity in this respect than occurred in reality. (See footnote under "Morphology and pleomorphism.")

Culture.	Temperature.	Dilution of serum.	Result.	Remarks.
"A" -----	28	1:10	+	Complete in thirty-five minutes; small, compact clumps. ¹
"City moat" -----	28	1:10	+	Complete in thirty minutes. ¹
"Pfeiffer" -----	28	1:10	+	Complete in thirty-five minutes; small, loose clumps. ¹
"554b" -----	28	1:10	-	Negative in thirty-five minutes.

¹ The hanging drop was not examined during the five minutes previous to the given time, hence it is probable that the table indicates a greater uniformity in this respect than occurred in reality. (See footnote under "Morphology and pleomorphism.")

The normal blood of a control rabbit gave no agglutination at 1:10 in forty-five minutes at 28°.

All of these cultures have been grown upon artificial media for from six to twelve months, with the exception of "Pfeiffer," which, as already stated, has been grown on artificial media for the past nine years. Cholera "Pfeiffer" agglutinates almost immediately at a 1:40 dilution, whereas it takes several minutes to produce complete results with the other cultures at this dilution. This susceptibility is not noticeable at the higher dilutions.

PFEIFFER'S REACTION (PERFORMED IN VITRO AFTER THE METHOD OF BORDET).

A loopful of the sodium chloride suspension of the culture to be tested was mixed with a loopful of the above-mentioned immune rabbit serum; a loopful of this mixture then added to a loopful of normal rabbit serum and the result watched in the hanging drop. All of the cultures, with the exception of "554b," agglutinated, the rods became swollen and globular, and in about three hours at 28° began to break up into granular masses; "554b" agglutinated, the rods became swollen but did not disintegrate. In control drops of immune serum alone the rods agglutinated, but no bacteriolysis occurred. In control drops of normal serum the rods retained their motility for three hours.

VI. THEIR PATHOGENICITY.

GUINEA PIGS.

In order to save guinea pigs for other purposes, only the pathogenicity of culture "579" toward these animals has been tested. At the time of isolation about 2 cubic centimeters of a twenty-four-

hour bouillon culture injected intraperitoneally killed a fair-sized guinea pig within twenty-four hours. Eleven months later, after direct passage through three guinea pigs, one loop¹ of a twenty-four-hour +1 agar culture, grown at 35°–37°, killed a 482-gram guinea pig in four hours. The peritoneal and thoracic cavities showed intense congestion with sero-sanguineous extravasations. The small intestine was greatly congested (much more so than the large) and filled with a yellowish mucoid fluid containing many desquamated epithelial cells, but, microscopically, no cholera spirilla. The abdominal organs were bound together by a fibrinous exudate. Pure cultures were obtained from the peritoneal cavity on agar plates. There were no organisms in the heart's blood.

PIGEONS (FULL GROWN AND OF ABOUT THE SAME SIZE).

Cultures "579," "Scout," "City moat," and "561" were pathogenic when one loop of a twenty-four-hour –1 agar culture suspended in salt solution was injected deep into the pectoral muscle. One loop of culture "A" failed to kill a pigeon. Five loops (about 30 milligrams) of culture "Pfeiffer" failed to kill. Abstracts of the protocols are as follows:

Pigeon 1.—One loop of "579" deep in left pectoral muscle. Dead in fifty-four hours. Congestion of cutaneous and deep vessels of left side. Cloudy swelling of left pectoral. Intestines congested. Microscopically, many curved rods in left pectoral, none in heart's blood. Many Halteridia and shadow corpuscles in blood. Pure cultures were obtained from the left pectoral muscle and heart's blood (three colonies per loop), which agglutinated with the "579" immune rabbit serum at 1:200 in about twenty minutes.

Pigeon 2.—One loop of "Scout" deep in left pectoral; dead in twenty hours; tissue changes as in Pigeon 1; organisms present in pectoral and heart's blood microscopically; many Halteridia present; pure cultures from pectoral and heart's blood which agglutinated with "579" rabbit serum at 1:200.

Pigeon 3.—One loop of "City moat" deep in left pectoral; dead in thirty-four hours; tissue changes similar to first case; organisms numerous at seat of injection, not found microscopically in heart's blood, which contained numerous Halteridia and many shadow corpuscles. Pure cul-

¹The same loop was used throughout the following experiments. When it holds just sufficient culture to fill the cavity of the loop and form a rounded surface on each side, its contents weigh 7 milligrams (wet). Allowing 1 milligram for loss during manipulation "one loop" signifies about 6 milligrams of the culture.

tures obtained from pectoral and heart's blood, which agglutinated with "579" rabbit serum at 1:200.

Pigeon 4.—One loop of "561" deep in left pectoral; dead in forty-four hours; tissue changes as above; curved rods at site of injection and quite a number in the heart's blood; few Halteridia; cultures from pectoral and heart's blood pure and agglutinate with "579" rabbit serum at 1:200.

Pigeon 5.—One loop of "A" deep in left pectoral; alive and well on tenth day; blood from foot shows very few Halteridia.

Pigeon 6.—One loop of "Pfeiffer" deep in left pectoral; alive and well on tenth day. No Halteridia found in blood from foot.

Pigeon 7.—Two loops of "Pfeiffer" deep in left pectoral; alive and well on sixth day. No Halteridia found in blood from foot.

Pigeon 8.—Five loops of "Pfeiffer" deep in left pectoral; alive and well on fourth day; blood from foot shows a number of Halteridia.

The dose injected into these pigeons seems to be rather large, but was adopted on account of the age of the cultures. I have not been able to test the relative resistance of a pigeon showing marked Halteridium infection on the one hand and one free from it on the other hand, on account of the difficulty of obtaining uninfected pigeons. Culture "Pfeiffer" has been grown on artificial media for such a great length of time that it could hardly be expected to be pathogenic except in large doses. (See footnote under "Morphology and pleomorphism.")

Monkeys.—Several attempts to infect monkeys by feeding have been performed with negative results, but I have notes on one case only.

An old adult male monkey (*Macacus cynomolgus*) received the contents of a recent agar slant culture of "579" suspended in — 1 bouillon. This was injected by means of a catheter into the stomach. He remained perfectly well for twenty-four hours. During the next four days 35 cubic centimeters of native spirits, called "arac" (containing about 40 per cent alcohol), were injected into his stomach. During part of the time he appeared to be intoxicated and refused to eat. Five cubic centimeters of 1 per cent sodium carbonate was injected into his stomach, followed by the contents of three + 1 agar slants of "579" suspended in — 1 bouillon. He did not vomit; faeces were normal, and he remained well during a week's observation. The culture "579" had been grown on artificial media for nine months without passage through an animal.

VII. THEIR MORPHOLOGY AND PLEOMORPHISM.

Each of these cultures shows that tendency toward pleomorphism, which is quite as marked as that seen in cultures of *B. pestis* or *B. mallei*, and which is so confusing to the beginner.

It is generally admitted that no two separate lots of media, which are identical in composition and reaction, can be prepared; and though the methods recommended by the American Committee, and the somewhat modified ones employed in these experiments give

comparable results so far as the reaction may indicate uniformity in composition, some bacteria will point out variations not detectable in other ways. I have not been able to reproduce exactly the same type of morphology in any two successive cultivations of the same culture, even on agar from the same batch, although precautions were taken to grow the cultures under apparently identical conditions, to make the preparations from corresponding portions of the growth, and to subject them to, as nearly as could be judged, like conditions of heating, staining, etc. On agar made on separate occasions the variation is still more marked.

In the case of the cholera spirillum which reaches its maximum growth on moist agar in such a short time, variations in morphology will be shown in preparations from the same culture. Thus, one made from the *edge* of a streak on an agar slant, where the younger forms are still multiplying, may present an entirely different appearance from one made from the *center* of the streak, where the older forms have lengthened out and are undergoing involution changes, as shown in figs. 7 and 8.

The variation which is so striking in this instance is not always so apparent in any of the cultures under consideration, nor is it appreciable in a twenty-four-hour culture of the less pleomorphic *B. coli*. Still this difference in the morphology of the younger and older forms of the cholera spirillum must be taken into consideration in comparing the morphology of different cultures, and when it is taken into account the variation on agar from the same batch may not be so marked.¹ (Compare figs. 8, 9, 10, 11, 12.)

It is hardly necessary to say that no permanent variations in morphology were produced in any culture. Figure 5 represents one which was kept in bouillon for ten months. This culture has been described under "Pellicle production in fluid cultures" as "579A." It will be noted that the organisms are somewhat larger than those pictured in figs. 2, 3, and 4. This is but a temporary modification, transmitted while the culture is kept in bouillon, but

¹ It seems worthy of note that in the preparation of the saline emulsions, for the agglutination and animal inoculation experiments heretofore cited, no such precautions were taken. It seems that the failure to do so may account for some of the variations observed. It is not at all impossible, for example, that a "loop" of young healthy cholera spirilla, taken from the edge of the growth on an agar slant, would exert a greater pathogenic action than one taken from the center where the growth is composed of old semidegenerate individuals.

very soon reverting to the shorter, thinner type when grown in peptone solution or transplanted upon +1 agar.

Figure 6 shows some of the long straight and spiral threads which grow out in the pellicle which forms on a liquefied gelatin culture. These are undoubtedly involution forms, for when a portion of such a pellicle is transplanted upon +1 agar, the short curved forms ordinarily met with upon agar develop abundantly. Further, in stained preparations from such an agar surface these threads and spirals take the stain poorly, and after two or three transplants disappear entirely.

VIII. SUMMARY AND CONCLUSION.

(1) The substance of this article consists, essentially, in making a careful preliminary study of the variations which occur in one culture of the cholera spirillum, and then comparing it with cultures from different sources.

(2) Certain reasons are given for adopting a modification of the methods of neutralizing media recommended by the American Committee—the hydrogen ion being left out on account of its toxic action.

(3) The cholera spirillum is not a nitrifying organism, and the successful demonstration of the “cholera-red reaction” in a solution of Witte’s “peptone” depends upon the presence of a trace of nitrates. Certain reasons are given for presuming that a variation in the nitrate content of media exists.

(4) The type of liquefaction produced in gelatin is influenced to a marked degree by the reaction and melting point of the gelatin. Sodium carbonate does not exert a more favorable influence on the proteolytic activity of the cholera spirillum than sodium hydroxide—at least so far as the liquefaction of gelatin is concerned. The proteolytic activity of a culture could not be increased by passage through a series of gelatin tubes.

(5) The optimum condition for growth is furnished by an albuminous medium containing between 1/50 and 1/100 of a gram-molecule of NaOH or Na_2CO_3 per liter, and this corresponds fairly well with the optimum conditions for the tryptic digestion of fibrin.

(6) Alkali, detectable by titration with phenolphthalein, is not produced in sugar-free bouillon devoid of sodium chloride.

(7) Growth in the presence of carbohydrates reveals that the acids produced from glucose, maltose, and saccharose rapidly kill

the cholera spirillum, while those produced from lactose and starch are not toxic—at least within a given time.

(8) The cultures studied are specifically the same as shown by the Gruber-Durham and Pfeiffer reactions. In order to obtain comparable results, quantitative variations between the agglutinin and agglutinable substance were excluded as far as possible.

(9) The pathogenicity for guinea pigs, pigeons, and monkeys is mentioned.

(10) Upon comparing the morphology of the different cultures it was noted that if precautions be taken to make preparations from corresponding portions of the growths, the variations were not so marked.

In conclusion, I wish to express my gratitude to Dr. Paul C. Freer, Superintendent of Government Laboratories, for many valuable corrections of the chemical concepts put forth in this paper and for many helpful suggestions in its editing.

DESCRIPTION OF PHOTOGRAPHS AND PHOTOMICROGRAPHS.¹

FIG. 1. Showing the variation in the amount and type of liquefaction produced in forty-eight hours in gelatin of varying reaction and dryness. The +1.5 tube has not photographed well; it showed no liquefaction along the stab and but a small, circular liquefied depression at the surface.

2. Coverslip preparation from the human ileum showing the morphology of the organisms of culture "579" as seen scattered about among the desquamated epithelial cells. (The photograph has been carefully retouched by the photographer in order to aid reproduction.)
3. Culture "579." preparation from the edge of a +1 agar slant, grown for twenty-two hours at 35°-37°.
4. Culture "579." from just below the pellicle on +1 bouillon; twenty-four hours at 35°-37°.
5. Culture "579.A." from the pellicle on +1 bouillon; twenty-four hours at 18°-25°.
6. Culture "579." from the pellicle formed on +1 gelatin; five days at 18°-25°.
7. Culture "Scout." from the *edge* of an eighteen-hour culture on +1 agar; at 35°-37°.
8. Culture "Scout." from the *center* of the same growth from which fig. 7 was prepared; showing older and involution forms.
9. Culture "561." from the *central* portion of a twenty-hour culture on +1 agar; at 35°-37°.
10. Culture "A." from the *central* portion of a twenty-hour culture on +1 agar; at 35°-37°.
11. Culture "city moat." from the *central* portion of a twenty-hour culture on +1 agar; at 35°-37°.
12. Culture "Pfeiffer." from the *central* portion of a twenty-hour culture on +1 agar; at 35°-37°.

¹Taken by Charles Martin, photographer. Bureau of Government Laboratories. (X 880.)

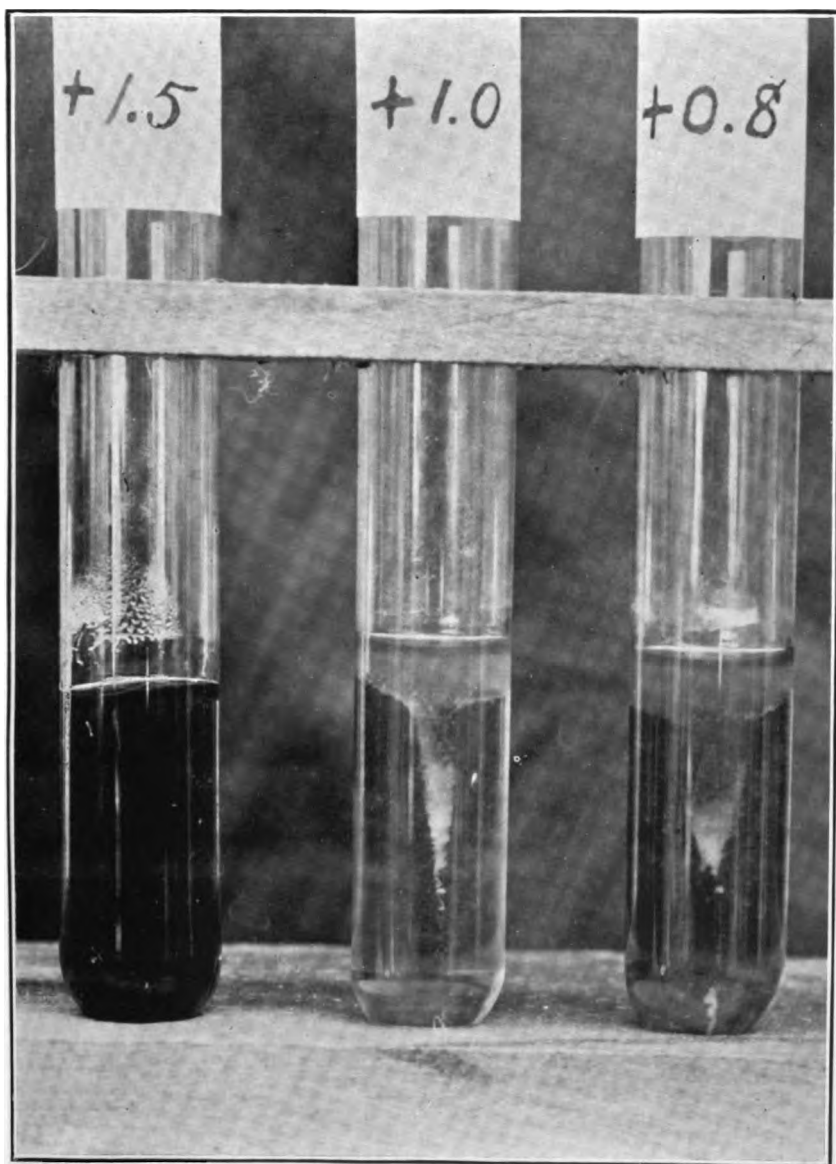


Photo by Martin.

Fig. 1.

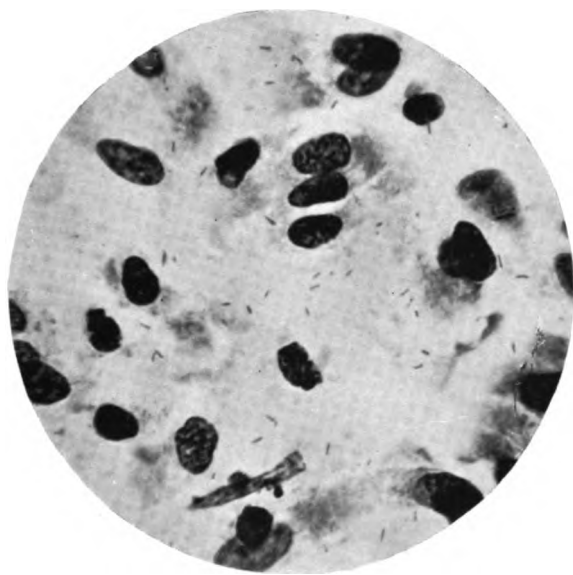


FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.

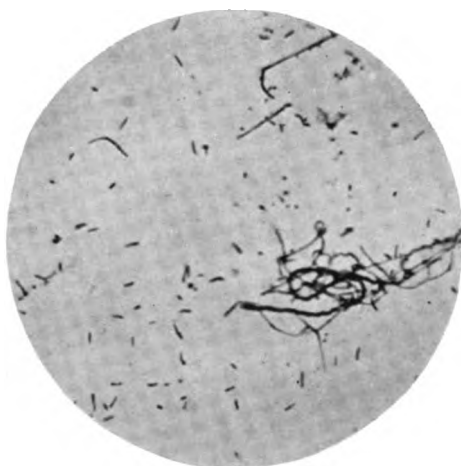


FIG. 6.

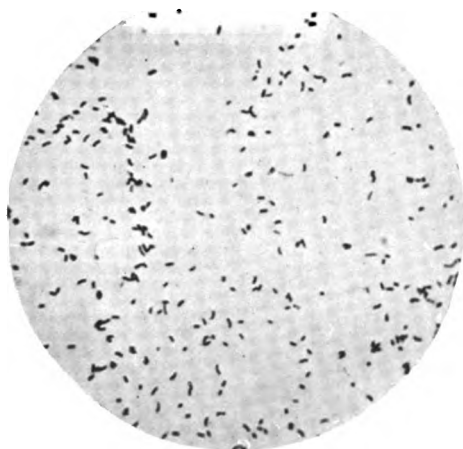


FIG. 7.



FIG. 8.

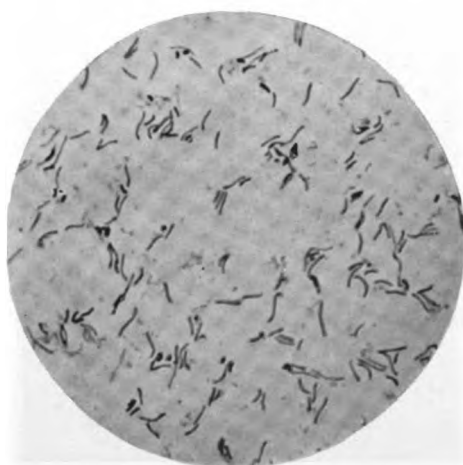


FIG. 9.

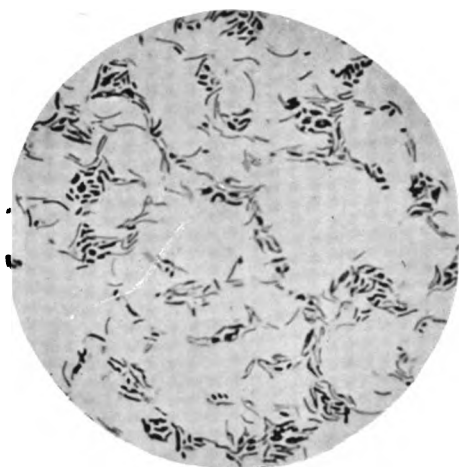


FIG. 10.

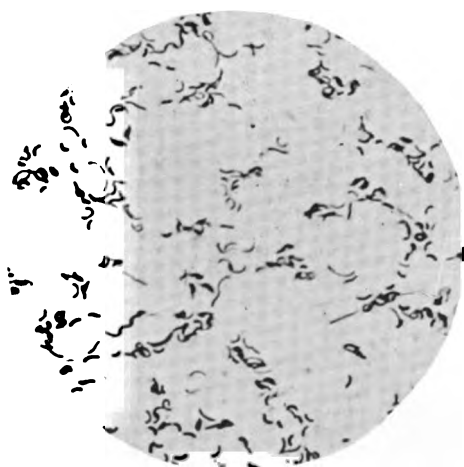


FIG. 11.

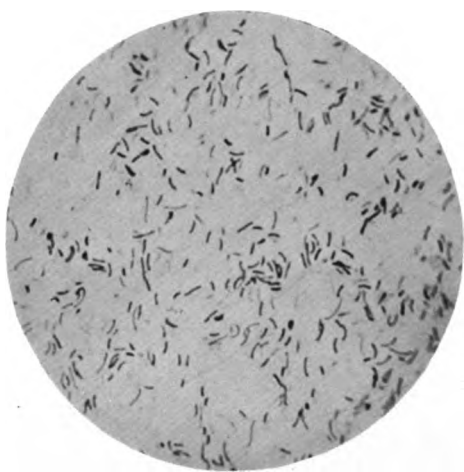


FIG. 12.

PREVIOUS PUBLICATIONS OF THE BUREAU OF GOVERNMENT LABORATORIES.

- No. 1, 1902, *Biological Laboratory*.—Preliminary Report of the Appearance in the Philippine Islands of a Disease Clinically Resembling Glanders. By R. P. Strong, M. D.
- No. 2, 1902, *Chemical Laboratory*.—The Preparation of Benzoyl-Acetyl Peroxide and Its Use as an Intestinal Antiseptic in Cholera and Dysentery. By Paul C. Freer, M. D., Ph. D.
- No. 3, 1903, *Biological Laboratory*.—A Preliminary Report on Trypanosomiasis of Horses in the Philippine Islands. By W. E. Musgrave, M. D., and Norman E. Williamson.
- No. 4, 1903, *Serum Laboratory*.—Preliminary Report on the Study of Rinderpest of Cattle and Carabaos in the Philippine Islands. By James W. Jobling, M. D.
- No. 5, 1903, *Biological Laboratory*.—Trypanosoma and Trypanosomiasis, with Special Reference to Surra in the Philippine Islands. By W. E. Musgrave, M. D., and Moses T. Clegg.
- No. 6, 1903.—I. New or Noteworthy Philippine Plants. II. The American Element in the Philippine Flora. By Elmer D. Merrill, Botanist.
- No. 7, 1903, *Chemical Laboratory*.—The Gutta Percha and Rubber of the Philippine Islands. By Penoyer L. Sherman, Jr., Ph. D.
- No. 8, 1903.—A Dictionary of the Plant Names of the Philippine Islands. By Elmer D. Merrill, Botanist.
- No. 9, 1903, *Biological Laboratory*.—A Report on Hemorrhagic Septicemia in Animals in the Philippine Islands. By Paul G. Woolley, M. D.; and Jas. W. Jobling, M. D.
- No. 10, 1903, *Biological Laboratory*.—A Report on Two Cases of a Peculiar Form of Hand Infection Due to an Organism Resembling the Koch-Weeks Bacillus. By John R. McGill, M. D., and Wm. B. Wherry, M. D.
- No. 11, 1903, *Biological Laboratory*.—Entomological Division, Bulletin No. 1. Preliminary Bulletin on Insects of the Cacao. Prepared especially for the benefit of farmers. By Chas. S. Banks, Entomologist.
- No. 12, 1903, *Biological Laboratory*.—Report of Some Pulmonary Lesions Produced by the Bacillus of Hemorrhagic Septicemia of Carabaos. By Paul G. Woolley, M. D.
- No. 13, 1904, *Biological Laboratory*.—Fatal Infection by a Hitherto Undescribed Chromogenic Bacterium: Bacillus Aureus Foetidus. By Maximilian Herzog, M. D.
- No. 14, 1904.—*Serum Laboratory*: Texas Fever in the Philippine Islands and the Far East. By Jas. W. Jobling, M. D., and Paul G. Woolley, M. D. *Biological Laboratory*: The Australian Tick (Bophilus Australis Fuller) in the Philippine Islands. By Charles S. Banks, Entomologist.
- No. 15, 1904, *Biological and Serum Laboratories*.—Report on Bacillus Violaceus Manila, a Pathogenic Micro-Organism. By Paul G. Woolley, M. D.
- No. 16, 1904, *Biological Laboratory*.—Protective Inoculation Against Asiatic Cholera: An Experimental Study. By Richard P. Strong, M. D.
- No. 17, 1904.—New or Noteworthy Philippine Plants. By Elmer D. Merrill, Botanist.
- No. 18, 1904, *Biological Laboratory*.—I. Amebas: Their Cultivation and Etiologic Significance. By W. E. Musgrave, M. D., and Moses T. Clegg. II. The Treatment of Intestinal Amebiasis (Amebic Dysentery) in the Tropics. By W. E. Musgrave, M. D.
- No. 19, 1904, *Biological Laboratory*.—Some Observations on the Biology of the Cholera Spirillum. By W. B. Wherry, M. D.

[In press. Edition of 2,000.]

- No. 21, 1904, *Biological Laboratory*.—Some Questions Relating to the Virulence of Micro-Organisms with Particular Reference to Their Immunizing Powers. By Richard P. Strong M. D.

(Continued on third page of cover.)

No. 20.—OCTOBER, 1904

DEPARTMENT OF THE INTERIOR
BUREAU OF GOVERNMENT LABORATORIES

BIOLOGICAL LABORATORY

I. DOES LATENT OR DORMANT PLAGUE EXIST
WHERE THE DISEASE IS ENDEMIC

By MAXIMILIAN HERZOG, M. D., AND CHARLES B. HARE

SERUM LABORATORY

II. BRONCHO-PNEUMONIA OF CATTLE: ITS
ASSOCIATION WITH B. BOVISEPTICUS

By PAUL G. WOOLLEY, M. D., AND WALTER SORRELL, D. V. S.

III. REPORT ON PINTO (PAÑO BLANCO)

By PAUL G. WOOLLEY, M. D.

CHEMICAL LABORATORY

IV. NOTES ON ANALYSIS OF THE WATER FROM
THE MANILA WATER SUPPLY

By CHARLES L. BLISS

SERUM LABORATORY

V. FRAMBOESIA: ITS OCCURRENCE IN NATIVES
OF THE PHILIPPINE ISLANDS.

By PAUL G. WOOLLEY, M. D.

MANILA
BUREAU OF PUBLIC PRINTING
1904

22994

LETTERS OF TRANSMITTAL.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,
Manila, P. I., September 30, 1904.

SIR: I have the honor to transmit herewith, for publication in one bulletin of the Bureau of Government Laboratories, the following: I. Does Latent or Dormant Plague Exist Where the Disease is Endemic? II. Broncho-Pneumonia of Cattle: Its Association with *B. bovissepticus*. III. Pinto (Paño Blanco). IV. Notes on Analysis of the Water from the Manila Water Supply. V. Framboesia: Its Occurrence in Natives of the Philippine Islands.

I am, very respectfully,

PAUL C. FREER,
Superintendent of Government Laboratories.

HON. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY, OFFICE OF THE DIRECTOR,
Manila, P. I., July 15, 1904.

SIR: I have the honor to transmit herewith and to recommend for publication a paper entitled "Does Latent or Dormant Plague Exist Where the Disease is Endemic?" by Dr. Maximilian Herzog, Pathologist Biological Laboratory, and Mr. Chas. B. Hare, Assistant Bacteriologist.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

DR. PAUL C. FREER,
Superintendent Government Laboratories, Manila, P. I.

PART I.

DOES LATENT OR DORMANT PLAGUE EXIST WHERE THE DISEASE IS ENDEMIC?

By MAXIMILIAN HERZOG, M. D., *Pathologist Biological Laboratory*, and
CHAS. B. HARE, *Assistant Bacteriologist*.

On August 21, 1903, Mr. Henry A. Blake, (1) governor of Hongkong, addressed a communication to the secretary of state for the colonies, entitled "Bubonic Plague in Hongkong: Memorandum by His Excellency the Governor, on the Result of the Treatment of Patients in Their own Houses and in Local Hospitals during the Epidemic of 1903." The writer of the memorandum makes some very startling assertions as to the danger of the spread of plague by animals of the most varied kind, and also comes to the amazing conclusion that there existed in Hongkong during the period of time intervening between June 23 and July 10, 1903, between 8,000 and 9,000 or more individuals among the native population in which plague bacilli were present in the circulating blood in spite of the absence of all clinical symptoms of the disease. The governor calls this condition "latent plague" and considers it a potent factor in the spread of the infection, and a factor which can not of course be reached by the ordinary methods employed to limit the spread of and possibly to suppress plague.

Fully to understand the statements of the governor of Hongkong, it will be well to quote a few paragraphs from his memorandum, which read as follows:

We have from Professor Simpson's report evidence that pigs, calves, sheep, monkeys, geese, ducks, turkeys, hens, pigeons, and rats are susceptible to plague, which may be contracted by food or by inoculation direct,

or by means of suctorial insects. To this list the examination mentioned above adds bugs, spiders, flies, and cockroaches. I may add that quails kept in the market for sale were also found to be infected. In paragraph 22, page 100, Professor Simpson points out that domestic animals suffer from chronic plague, and surmises that this is probably one of the bridges by which the interval of the attacks in man is connected. I have for a considerable time been of the opinion that man himself is subject to chronic plague, which may either pass away after a considerable time or continue dormant over the winter months, regaining activity with the annual movement of spring, when the curve of the epidemic is almost constant. This opinion was strengthened by the fact that in August, 1899, the body of a Chinese lift man at Queen's buildings who was accidentally killed when attempting to enter the lift while in motion was found to contain plague bacilli. A similar result followed the examination of a man who on the 4th of March, 1901, was killed at Tal Koo Sugar Works by a bag of sugar falling on his head from a height of 20 feet; while on the 2d of April, 1903, in the body of the chief steward of a ship lying in the dock, found floating with a large wound on the head, were also found plague bacilli. Early in June several men from H. M. S. *Ocean* were sent to the naval hospital, suffering from pneumonia; on examination of their blood seven were found to be suffering from mild cases of plague. In like manner two officers of the Sherwood Foresters who developed feverish symptoms were, on having their blood examined, found to be similarly affected. In the "Boletim Oficial" of Macao, containing the report on the plague epidemic, 1895, Dr. Gomes de Silva, the medical officer who published the report in 1895, stated that during the height of the epidemic he had discovered plague bacilli in his own excreta.

(21) In June I directed Inspector Gidley to obtain as many specimens of blood as possible, on slides secured from the Government bacteriologist. He obtained 110 specimens from men, women, and children taken at random. These slides were sent to Dr. Hunter for examination, who reported that in five slides he found plague bacilli, and in seven slides bacilli were present in considerable numbers, some of which showed bipolar staining. They were not sufficiently distinctive, however, to be regarded as *B. pestis*. These slides were obtained between the 23d of June and 10th of July. Since they were obtained there were but three cases of plague in the district, from none of which a specimen of blood was taken.

(22) I am not unmindful of the fact that these reports were the result of microscopic examination only. But the examination was the same as that on which a great many of the cases treated in the Kennedy Town Hospital were sent to that institution where their cases ran the usual course of plague invasion.

(23) Now, putting aside the seven doubtful slides, it will be seen that of those people examined at random 4.54 per cent were found to be infected with plague though to all appearances perfectly healthy. If we exclude all the well-to-do, and take the working coolie population alone, they

probably number 180,000, and, assuming the same average amount of infection, there are among that class alone 8,172 persons at present infected in Hongkong. If even a quarter of that average be accepted for the 105,000 inhabitants of the superior class the number of infected will be increased to 9,634. In Appendix G¹ will be found the number of rats examined in each month of the present year with the proportion of the infected rats. I am afraid that the incidents mentioned in paragraph 5 weakens deduction as regards Hongkong. But, from whatever source the rats were procured, the proportion of infection in June was 9 per cent or 4.46 per cent more than the percentage of the slides examined, or, if doubtful cases mentioned by Dr. Hunter be included, 1 per cent less, while in January the proportion falls to 0.8 per cent. This being so, with the complete circle of vermin, insects, food, rats, domestic animals, and man all infected in possibly similar—possibly different—proportion, it appears to me unsound to concentrate attention upon the rat as the principal means of bridging over the dormant season.

It appears that Governor Blake, after writing the above, felt the great danger of coming forward with so sweeping an assertion, and in the introduction to his memorandum he himself makes an appeal for a more thorough scientific investigation of the hypothesis of the existence of latent or dormant plague among the natives of countries where this disease is endemic. He says:

My hypothesis in paragraph 23 may not bear the light of scientific investigation, and, as the hypothesis of a layman, may not carry much weight, but I venture to submit that it is worthy of scientific inquiry, for while a timely glass of water may prevent a great conflagration, and plague at its first introduction may be stamped out by immediate segregation and thorough disinfection, its endemicity once established this is no longer practicable, and, if the hypothesis of dormant or chronic plague in man be ultimately proved to be correct, it is difficult to see how quarantine for even ten days can prevent its annual recurrence, or how any practicable examination of departing passengers can prevent its export from the plague center and possible dissemination elsewhere if suitable conditions for its propagation be present. What the remedy or what the necessary precaution, I leave it for scientific men to determine, but if my hypothesis results in a wider radius of investigation the experiment will not have been useless.

THE RESULT OF BLOOD EXAMINATIONS IN CASES OF PLAGUE.

It is, of course, obvious to any one versed in examinations of this nature that a diagnosis of plague can not be made by a microscopic examination of the blood. Such an examination may possibly be

¹ Omitted in this bulletin.

resorted to in urgent situations, when a rapid clinical diagnosis is desired, but to base far-reaching deductions upon such a microscopic examination is certainly not permissible. What is necessary in order to determine beyond doubt the presence of plague bacilli in the circulating blood is the examination of the latter by cultural methods. We have undertaken a series of such examinations in order to determine whether or not there exists such a thing as latent or dormant plague, as suggested by the governor of Hong-kong. Before giving the details of these experiments it will be well to make a survey of the work that has been done with reference to the presence of plague bacilli in the blood in undoubted cases of this disease.

The German Plague Commission (2), in its report published in 1899, page 265, made blood examinations in the case of 141 plague patients, including 17 who were in the period of convalescence varying between the seventeenth and twenty-fifth day after the disease. These examinations were made in the following manner: A drop of blood was obtained from the finger, with the usual aseptic precautions, and inoculated into agar tubes, while at the same time a cover-glass preparation was also made. It was found that in a number of cases where the culture method furnished positive results, the mere microscopic examination failed to demonstrate the presence of the bacilli. Of 124 patients whose blood was obtained during the climax of the disease, in 81 even repeated examinations did not demonstrate the presence of plague bacilli, in 10 the bacilli were encountered only once, while in 33 they were repeatedly found in the blood. Of 81 patients the examinations of whose blood were always negative, 52 recovered and 29 died. Of 10 cases, in which there was a positive result only once, the other examinations being negative, 8 died and 2 recovered. It is interesting to note that in one the bacilli could be detected two and three days before death, while twelve hours before the crisis and at the post-mortem examination it was impossible to find them. Seventeen convalescent patients invariably failed to show any bacilli in the circulating blood.

Zobolotny (3), in his researches on plague, says that the bacilli are found in large numbers in the blood of animals sick with the disease, but that in the case of human beings the bacteria are much less abundant and sometimes can not be found at all.

Muschold (4), quoting the work of *Albrecht* and *Gohn*, reports that the latter examined 122 cases of undoubted plague. In 55 the bacilli were found by the culture method in the circulating blood during life. Four of these 55 cases recovered, and in 2 of them the bacilli were present in large numbers in the circulating blood. In the case of the 51 patients who died the bacilli were found in considerable numbers in the blood on the day of death as well as on the previous one.

Cairns (5) studied the blood of patients during an epidemic of plague occurring in Glasgow in 1900. He gives the results of examinations made *inter vitam* on cases which subsequently terminated fatally. Four of these may be cited in connection with our investigation. In three fluid drawn from the buboes developed pure cultures of *Bacillus pestis*, and only in one of the four fatal cases was it possible to obtain cultures of the bacilli from the blood during life. The other three cases gave negative results. In one of these, seven daily consecutive examinations were made, as well as one shortly before death, all of them proving negative.

Calvert (6) studied two epidemics of plague in Manila in 1900 and 1901. He found that the clinical examination of the blood for *Bacillus pestis* gave unreliable results, it being impossible to place any weight on the negative findings. He made his examinations by taking the blood from the ears of the patients at intervals of four hours, and examining it in smears as well as by the culture method. This plan was followed until the death or recovery took place. Thirty-six cases, 4 of which recovered, were examined in this manner, most of them being followed to autopsy, when the plague organism was demonstrated by culture and even by animal inoculation.

This author gives the following table of positive findings of the bacilli in the blood:

	Per cent.
24 hours before death in 31 cases.....	100
48 hours before death in 7 cases.....	22
72 hours before death in 5 cases.....	16.12
96 hours before death in 2 cases.....	6.45
120 hours before death in 1 case.....	3.22

On looking over the table it appears that the plague bacilli could be found in all fatal cases twenty-four hours before death, but that forty-eight hours before death the percentage of positive findings was much smaller. In searching for the bacillus in the blood of plague patients who finally succumb to the disease, the chances of finding the microbe five days before the fatal termination are only one out of thirty. All of this shows that even in fatal cases the plague bacilli are not found in the peripheral blood at an early date.

Jennings (7), in his *Manual of Plague*, page 112, says that it is extremely rare to find plague bacilli in the blood in large numbers except immediately before death. Their absence, therefore, in the early stages of an attack is frequent, and must not be regarded as a negative diagnosis of plague.

Terni (8), who studied the plague in Rio de Janeiro, in an excellent article on the disease makes the following statement:

"The examination of the blood is by no means reliable. It is astonishing that Galeotti places any value in blood examinations in the diagnosis of early plague."

Terni found that in many cases which were diagnosed as plague septicemia, examinations of the blood, both microscopic and by culture method, were negative. This was true even at the post-mortem examination, because the bacilli were exclusively localized in the lymph channels. Even in the most profound cases, in individuals particularly predisposed to the disease, the bacilli were found in the blood only in moderate numbers. Their presence could never be compared with what has been found to be true in connection with other septicemic microbes, such as anthrax or diplococci. A multiplication of the plague bacilli is found only in exceptional cases in the circulating blood during the agonal stage.

One of us has been studying for some time the morbid anatomy and histo-pathology of a number of cases of plague. These studies appear fully to confirm the statement of Terni to the effect that plague, as a rule, is not to be looked upon as a true septicemia, but, on the contrary, as an infection particularly confined to the lymphatic system. Even in cases where sections from the lymph glands contain innumerable bacilli, the lumina of the blood vessels are generally free from such microbes. In fact, in the study of sections from all of the internal organs when plague bacilli are seen they are always found in the lymph channels or lymph spaces and not in the blood vessels.

Powell (9) has recently reported the result of 3,400 blood examinations of febrile diseases in Bombay. Most of these cases were malaria, but 117 of them were plague. In only 15 of the latter were the bacilli easily seen in blood smears. With reference to the finding of the bacilli in the blood in cases of plague, the author says: "As regards the recognition of the plague bacilli in the finger blood, for some years I was very sceptical about the reports of certain medical men, and until within the past eighteen months had been unable to detect the bacilli except on cultures. At the beginning of this year there was a particular type of septicæmic plague, in which the bacilli were found in every case. Such cases in my experience always died. One case seemed to be convalescent and had a normal temperature for three days, but suddenly died. Plague bacilli were found eleven days before death."

The *Indian Plague Commission*, speaking of the bacteriologic diagnosis of plague by microscopic examination of suspected material, makes the following statements:

(156) In the case of blood derived from a patient who is suspected of suffering from plague, the detection of bacteria possessing the morphological characters of the plague bacillus will (especially if these are present in large numbers and when it is determined that these become decolorized by Gram's process) be conclusive evidence that the patient is suffering

from plague. The mere finding of a few isolated bacteria arranged together as diplococcal forms can not, especially when Gram's test has not been applied, be accepted as establishing the diagnosis of plague. We have in view here in particular certain suspected cases which occurred in Calcutta in 1896, in which it was claimed by Professor Simpson that the diagnosis of plague was confirmed by his bacteriological examinations. We would note with regard to these first that in some of the suspected patients only a few isolated diplococcal forms were found after long searching through a number of films prepared from the blood. Again, in view of some of the figures reproduced in the record of Dr. Simpson's evidence, particularly Figs. I, VI, C, and F, it is important to note that it does not seem to have been determined whether bacteria became decolorized when treated by Gram's process, and, lastly the fact must not be lost sight of that saprophytic diplococci of various kinds are widely distributed in nature, and that there is always a possibility of microscopical preparations containing as contaminations a small number of such diplococcal forms.

In looking over these statements (all that we can find in the literature at our disposal), one is certainly impressed with the fact that the finding of plague bacilli in confirmed cases of the disease, except very shortly before death or in the rarer cases of plague septicæmia, is the exception and not the rule. Indeed, we should be mindful of the fact that plague, as a rule, is not a septicæmia but a bacterial infection localized in the lymphatic system. It is, therefore, from a purely theoretical standpoint, highly improbable that there should exist a dormant, latent clinical form of the disease in which the patients harbor the bacilli in the circulating blood.

Our investigations to determine whether such is the case or not were made on a number of native Filipinos and Chinese of this city. We attempted to get material which, if latent plague exists at all, would give us some evidence to this effect, therefore we selected natives from houses in which plague cases had occurred. We examined a number of the inmates of Bilibid Prison, particularly such as were under the most unfavorable hygienic conditions, namely, insane prisoners and those of the third class who were most crowded in their quarters. Since we could not always get natives of this description, we selected also a number which were not under particularly unfavorable hygienic conditions, such as native police officers and native members of the Constabulary.

While plague has never at any time been as widespread here as in Hongkong, a sufficient number of cases have occurred to make

it clear that the disease is endemic, though fortunately not markedly epidemic.

According to the monthly sanitary reports of the Board of Health plague has prevailed in Manila since 1900 to the following extent:

Prevalence of plague in Manila, 1900 to 1904.

1900.

Month.	Chinese.	Filipinos.	Americans and other Caucasians.	Total number of cases.	Total number of deaths.
January.....	3	15		18	11
February.....	26	12		48	25
March.....	52	12		64	48
April.....	48	11		54	44
May.....	18	7	2	22	18
June.....	14	5		19	11
July.....	5	8		13	7
August.....	8	9	1	18	11
September.....	6			6	9
October.....	5	2		7	5
November.....	1			1	
December.....		1		1	
Total.....	186	82	3	271	199

1901.

January.....	4	3		7	5
February.....	15	11	1	27	20
March.....	49	14		63	51
April.....	73	38		111	91
May.....	97	40		137	124
June.....	24	30	1	55	54
July.....	18	20	1	39	38
August.....	12	16	1	29	26
September.....	7	4		11	12
October.....					
November.....					
December.....	1	4	1	6	6
Total.....	300	180	5	485	427

1902.

January.....					
February.....	1			1	1
March.....		1		1	1
April.....					
May.....					
June.....	1			1	1
July.....					
August.....	1			1	1
September.....	1			1	1
October.....		2		2	2
November.....	1			1	1
December.....		2		2	2
Total.....	5	5		10	10

Prevalence of plague in Manila, 1900 to 1904—Continued.

1903.

Month.	Chinese.	Filipino.	Americans and other Caucasians.	Total number of cases.	Total number of deaths.
January.....		1		1	1
February.....	7	10		17	15
March.....	18	15		33	38
April.....	35	15	2	52	49
May.....	16	9	2	27	23
June.....	9	23		32	25
July.....	3	11		14	9
August.....	10	1		11	9
September.....	3	1		4	4
October.....	3			3	2
November.....		2		2	2
December.....		2		2	2
Total.....	104	90	4	198	174

1904.

January.....	4	6		10	7
February.....		6	1	7	6
March.....	3	12		15	14
April.....	8	7		15	15
May.....	9	8		17	16
Total for five months.....	24	39	1	64	58

SUMMARY.

Reported in—	Plague cases reported.	Plague cases fatal.
1900.....	271	199
1901.....	485	427
1902.....	10	10
1903.....	198	174
1904 (Jan. 1 to May 31).....	64	58

It appears from these statistics that plague had completely died out during four of the months of 1902, since during this period not a single case came under observation. However, since August, 1902, until the time of writing the present report there has not been a month entirely free from plague, though the figures have generally been low, the maximum during this period being reached in April, 1903, when 52 cases of the disease were reported.

The object in giving these figures in connection with our report is to show that plague has been sufficiently prevalent here for several years, so that blood examinations should furnish evidence

of latent plague provided that such a form of the disease exists at all.

The figures of plague morbidity for Hongkong during the same years are as follows:

	Cases.
1900.....	1,086
1901.....	1,637
1902.....	540
1903.....	1,135

**BLOOD EXAMINATIONS OF 245 APPARENTLY HEALTHY NATIVE
FILIPINOS AND CHINESE.**

The method employed to ascertain whether apparently healthy Filipinos or Chinese of Manila have any plague bacilli in their blood was as follows:

The bend of the elbow, generally of the left arm, was very thoroughly cleansed first with strong alcohol and then with a strong solution of mercury bichloride, and finally with alcohol and sterile distilled water. A rubber bandage was then placed around the arm above the elbow and 1 cubic centimeter of blood was drawn from one of the veins by the aid of a sterile hypodermic syringe. The blood so obtained was added to 50 cubic centimeters of bouillon in a flask. The bouillon used was prepared as usual, and, when neutral to litmus, 0.5 gram of bicarbonate of soda was added to each 1,000 cubic centimeters of the bouillon, making it slightly, but decidedly, alkaline. This forms a very excellent culture medium for plague bacilli. As a control experiment in some of the cases about twice the amount of blood was drawn from the vein, and the 2 or 3 cubic centimeters so obtained was distributed in two flasks. One of the latter was then immediately inoculated with a very small amount of material from a young plague culture. This was, of course, done to see whether plague bacilli, if present, would develop in the bouillon in the presence of a small amount of freshly drawn blood. It may be said that the control flasks always developed a typical plague growth, so that evidently nothing in the arrangement of the experiment prevented development of the plague bacilli if any were present. The culture flasks to which blood had been added were kept either in a dark chest at room temperature or in an incubator which was fairly constant at 35° C. The media were inspected daily and when a growth developed it was examined in stained preparations and by culture on agar.

The native Filipinos whose blood was examined included 32 laborers from the Serum Institute and the morgue. The native servants of the latter, where all the plague post-mortem examinations of the city are made, are, of course, particularly exposed to infection, and would be especially prone to show latent plague provided that such a condition exists. These 32 cases were kindly examined for us by Dr. E. H. Ruediger, bacteriologist in the Serum Institute, whose technique differed from the method generally used only in that he employed a 5-per-cent carbolic acid solution to sterilize the elbow. Dr. Ruediger also examined all of his 32 flasks by stained cover-slip preparations and by culture methods whether they showed any change in appearance or not.

The blood examinations in all of the 245 cases were made between March 4 and May 20, 1904—i. e., during a period when from thirty-five to forty cases of plague were reported in Manila.

The following is a summary of the examinations:

On March 4 there were examined 5 native Filipinos from a house in Santa Cruz in which an ambulatory case of plague terminating in embolism of the pulmonary artery had occurred. Specimens were taken from 10 native Filipino police officers on April 6 and 7. Nine of these men lived in infected districts—i. e., those in which cases of plague had recently occurred; 1 came from a noninfected district. Ninety native Filipinos, members of the Philippines Constabulary, were examined between April 13 and 30. These men live in barracks, but are often free to visit their families and friends. During the first week in May 32 native Filipinos, laborers at the Serum Institute and the morgue, were the next subjects investigated. In the former place the various vaccines and sera, including plague vaccine and serum, are prepared, and in the latter are performed all the necropsies on plague cases. Fifty-eight native Filipinos, prisoners in Bilibid Prison, were taken on May 12 and 13. In this place about 4,500 men are confined. Last year a number of the inmates died from pneumonic plague, but during this one no case has occurred there, although one of the prisoners died of pulmonary plague four days after his discharge. Of these 58 men 16 were insane prisoners, all in advanced stages of degenerative mental disease, and the remainder were the so-called third-class prisoners, who lived under the most unfavorable conditions to be found in the prison.

On May 16 to 20 there were examined 50 Chinese, small shopkeepers, clerks, and coolies, either from houses in which plague cases (in one instance two such cases) had occurred or from those adjoining.

RESULTS OF THE EXAMINATION.

Most of the flasks to which 1 cubic centimeter of blood had been added remained sterile; although a few developed growths which,

however, were clearly contaminations from the air, such as common molds or similar forms of life. One of the cases taken by Dr. Ruediger developed *Staphylococcus pyogenes aureus*; and those of two other natives developed a bacillus which, when examined in a stained cover-glass preparation, might possibly be mistaken for *Bacillus pestis*. One of these organisms, however, in culture looked very different from the bacillus of plague and also retained Gram's stain. This bacillus developed in a flask to which had been added blood from one of the insane prisoners. The other growth occurred in a flask containing blood from a member of the Constabulary. This organism also greatly resembled morphologically the plague bacillus, but it kept Gram's stain, and when rubbed in large amounts on the shaved abdomen of a guinea pig failed to produce disease. In short, not in a single instance out of 245 examinations of persons of whom a large percentage had certainly been greatly exposed to plague infection did we find any evidence of the existence of plague bacilli in the blood.

CONCLUDING REMARKS.

From our investigations conducted on 245 native Filipinos and Chinese it may be safely concluded that a condition of latent or dormant plague does not exist in Manila, and there is hardly any reasonable doubt that it does not exist in Hongkong. There certainly has not been furnished the slightest proof of such a character as to stand the searchlight of exact methods of bacterial investigation to indicate that there is such a thing as latent human plague, with the presence of plague bacilli in the circulating blood, in the absence of clinical symptoms of the disease.

The governor of Hongkong himself, in his memorandum, clearly sets forth some of the circumstances which unite to make it practically impossible to completely eradicate the disease in Victoria City.

In Hongkong (1) (memorandum, par. 3) it is the custom of the Chinese—if they can possibly do so—to dump human corpses dead from plague into the street, in order that they may not be found in the houses and thereby subject the inmates to quarantine, disinfection, etc. In spite of measures to prevent this procedure, the number of corpses dead from the plague and so disposed of has, during the ten years preceding 1898, increased from 25.1 per cent to 32.7 per cent.

The Chinese in Hongkong, according to the governor's memorandum, offer passive resistance to the catching of rats in their houses, being afraid that plague bacilli might be found in the rodents, as this would lead to measures of disinfection or to the repair of their houses. The Chinese rat catchers are said to be dishonest; they fail properly to label the rats, so that infected houses escape detection, and they import rats from the outside of Hongkong and label them at random. In general they are very unreliable in their work and are actuated solely by the desire to secure from anywhere the largest number of rats in order to obtain the premium offered for each.

To those who know how Chinese houses are constructed [says the memorandum, in par. 6], it will be apparent that effective fumigation is practically unattainable. While, even if the spraying process, scrubbing and disinfection of clothing reached externally everything in the room, it would not kill vermin lying deep in the joints and cracks of the tables, chairs, and settees, or beds. Nor would it reach the vermin with which the heads of the poorer classes of coolies are infested. But apart from this, what took place in many cases when a case of plague was detected was that before the constable could arrive to take charge of the house, goods liable to injury by disinfection were removed by the door, or, if too late for this, were taken on to the roof, always easily accessible, and deposited in some neighboring house.

In W. J. Simpson's report (11) "On the Causes and Continuance of the Plague in Hongkong, etc.," which is quoted in the memorandum of the governor, we find the following statements as to the sanitary conditions in the Chinese quarters in the city of Victoria.

When a case of plague has once occurred in a house, there is a great tendency in subsequent years for the same house, or that adjoining, or that on the opposite side, or that close by, to be attacked with plague. When plotted out on a map, the distribution of plague appears to be closely connected with previous infection of the house or of a defined locality, the infection having been retained in an unrecognized form in the interval. The houses which suffer principally are, speaking generally, the most insanitary and the oldest. It has already been mentioned, how closely packed the buildings are in the older portions of the town, narrow streets and high houses being the leading features, by which the admission of sunlight and fresh air is considerably obstructed. Narrow streets and high houses, however, are not peculiar to Hongkong; they are to be found in other towns, with their injurious effects on health, but in Hongkong there is moreover in the Chinese quarters a defect in the construction of the houses which intensifies the obstruction of light. The rooms are long and narrow, with a window at each end, the front

window looking into a wide and covered veranda, and the back window into a small open space at the back, which forms a sort of wall between the two houses. The lower floors of many of the houses are remarkable for their darkness, and this in a region not far from the Tropics; they are also frequently damp.

Since the epidemic of 1894 many of the lower floors of the worst kind have been changed into storerooms to contain the goods and merchandise for which Hongkong is in entrepôt. These storerooms as a rule are infested with rats, which at times find their way up to the rooms on the higher floors. The basements are generally rat ridden, both floors and walls, and from the walls being often hollow it is easy for rats to reach the upper floors.

The admission of sunlight into the dwelling rooms of Chinese tenement houses is still further obstructed by the subdivision into several cabins or compartments, sometimes numbering up to six, which every room is subjected to. Each cabin is let out to a separate tenant and not infrequently accommodate a separate family. These compartments or cubicles are windowless rooms, and are often so dark that it is impossible for any one, coming directly from the light outside and drawing or opening the door of the cubicle, to see at once whether it is occupied * * *. Some attempts have been made to improve this state of things by limiting the height of the subdividing walls to six feet. The condition which obtained before this improvement has made it somewhat difficult to realize, for what I am describing is that which now exists. Fresh air and sunlight never get into the cubicles except perhaps the compartment at each end of the room opposite the window. The subdivision of a single room into a number of rooms called cubicles is an ingenious device for crowding together a large number of people into a small space and securing a correspondingly large rental, but it is an arrangement which engenders disease and favors its spread. There is no doubt whatever that every such windowless cubicle is unfit for human habitation and should not be permitted * * *. Probably another cause for the continuance of the plague, besides the insanitary condition of the houses referred to, is the very inadequate number of latrines and urinals with which Hongkong is provided. The number of public latrines appears to be twenty-nine belonging to the Government, and seventeen to private owners. The total number of seats is 1,202. Most of them have urinals attached, and in addition there are three small public urinals in the town. Seeing that all the men and boys go to the public latrines, there are no sanitary appliances in the houses except earthen pots, which are used exclusively by the women and children. The total inadequacy of the latrine accommodation provided is obvious. It is not one seat to one hundred of the male population. On the Kowloon side of the colony the latrine and urinal accommodation is still more deficient. Large blocks of houses have been built, and not a single latrine or urinal provided by the builder of the block. It is impossible under these conditions that the ground should escape being sewage polluted * * *. The existing latrines are far from being models

of what they should be. They are in fact insanitary in structure and deficient in light and ventilation.

Quite recently Surgeon-General Evatt, P. M. Q., His Majesty's troops, Hongkong, has been quoted by the daily papers in an interview in which he speaks in the strongest terms of the insanitary conditions, which he holds responsible for the continued prevalence of plague in Hongkong. He calls Victoria City the plague-distributing center of the world, a standing menace to the human race. So we have the most convincing evidence, both official and unofficial, as to the vicious sanitary conditions in Hongkong. These furnish the soil in which plague thrives, from which it can not be completely eradicated, and from which it breaks forth again and again in menacing epidemic form.

From our own investigation carried on in Manila, we have certainly good reasons to deny the existence of latent or dormant plague in our city. The statements of those who have looked into the conditions in Hongkong likewise appear unfavorable to the theory of the governor, and they offer an explanation more in accord with what we do know positively as to the nature of plague infection, and as to the spread of infectious diseases in general.

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PART II.

BRONCHO-PNEUMONIA OF CATTLE: ITS ASSOCIATION WITH *B. BOVISEPTICUS*.

By PAUL G. WOOLLEY, M. D., *Director of the Serum Laboratory*, and
WALTER SORRELL, D. V. S., *Veterinarian Serum Laboratory*.

The reason we care to dwell upon these cases is not that the above-mentioned pulmonary affection is uncommon or not well known, but because of its relation, here at least, to hemorrhagic septicæmia.

It is agreed by all authors that the causes of catarrhal pneumonia, in animals as well as in man, are not always the same. However, in general the chief ones are also those of acute bronchitis. Following this it happens, either because of swelling of the mucous membrane, or by aspiration of bronchial secretion, that the small subdivisions of the bronchi became plugged, and a condition of atelectasis arises in that part of the lung supplied by the affected bronchioles. Then because of the *locus minoris resistentiæ* furnished by the atelectatic part, and because of the usual presence of infectious material in the bronchial secretions and in the aspirated material, inflammatory processes occur, which may be limited or which may spread by continuity to the neighboring tissue. The changes following atelectasis may either be a gradual atrophy of the affected parts of the lung, or following infection, bronchiectasis, abscess formation, pleurisy, gangrene, etc., resolution, or calcification. The disease occurs especially in young animals and in older ones whose physical condition is below par. In young animals it may be epizootic.

The symptoms are said to vary. Schneidemühl says that the

first signs point to acute bronchitis, upon which ensue pulmonary symptoms, such as increased respiratory frequency and cough.

The course of the disease according to Diekerhoff and Schneidmühl is neither typical nor regular. Sometimes it progresses very rapidly. In other cases it may last for weeks or months.

In the more chronic ones a purulent pneumonia with pleuritis and pulmonary gangrene may ensue and death follow. It is in this last group, that of chronic cases, that ours belong.

These occurred in the herd of the Government Serum Laboratory. The calves were imported for use in the preparation of vaccine virus. The cattle were used in the preparation of antirinderpest serum. All of the calves originally came from China, and upon arriving in Manila had been treated with prophylactic doses of antirinderpest serum and kept under careful observation for several weeks before they were used for the intended purpose. At the time of vaccination there was no indication of disease in any member of the herd, their appetites were good, they were in excellent physical condition, and their temperature curves were normal. (Inasmuch as tuberculosis has never been observed in cattle here the vaccine calves are not slaughtered after collecting the virus, but are kept on hand until disposition can be made of them in some other way. As a rule, the physical condition of the animals improves after taking the vaccine. When this is not the case slaughter is resorted to when there is no particular reason, as in the present instances, of allowing the animals to live.)

The steer mentioned below was also imported from China for use in the preparation of antirinderpest serum, but its condition was never satisfactory for this purpose.

The histories follow:

CASE I.

Calf No. 437 was received at the laboratory on January 12, 1904, and received 100 cubic centimeters of antirinderpest serum at once. It remained apparently well with a normal temperature until January 24, when the latter rose to 40.7° C. The next day the temperature was 40.8° in the afternoon, and the following day it reached 41.2°. From this point it gradually declined to normal on January 30. On February 1, 5 cubic centimeters of virulent rinderpest blood was given to the animal, and on the third day after, it was vaccinated. Following this operation, there was what at first appeared to be the usual temperature of vaccinia, which reached 40.6° on the fifth day after vaccination. But this temperature instead of declining persisted with slight variations between 39° C.

and 41° C. until March 1, when it became normal and stayed so until death, March 24, 1904. On several occasions the blood of the animal was examined for trypanosomes with negative results.

During the course of the disease, the animal gradually lost weight in spite of a reasonably good appetite, and as it progressed the appetite became impaired and the coat rough and starchy. There was no cough, nor in fact any other notable symptoms than the gradual wasting.

The autopsy was made about twelve hours after death. The only appreciable lesions present were in the lungs. The other organs were in an apparently healthy condition, with the exception of the prescapular lymph glands, which were enlarged and edematous.

The apices of the lungs were chiefly affected. These portions of the organ were almost completely solidified. The surface of the hepatized portions were mottled with yellowish-white areas which stood out distinctly upon a reddish-purple ground. In palpating, the finger was able to detect that these lighter areas were firmer than the darker colored portions of the lungs, which had an edematous consistency. Over several of the nodules there was a thin, fibrinous membrane under which the pleura was congested and roughened. Upon section, the affected portions of the organs cut with increased resistance, and from the incisions a frothy serum oozed. The general color of the surface was dark red, mottled by the grayish yellow of the sometimes almost caseous nodules. Sections of the latter had all the macroscopic characteristics of the gray hepatized tissue of pneumonia, being granular and dry. In an occasional one the process had gone beyond the simple hepatization and the center had softened, producing a rather creamy material.

From several of such tubercles, cultures were made upon agar and in bouillon, and pieces of the tissue were placed in Zenker's solution, and absolute alcohol, for further study. Smears made from the pulmonary nodules showed a few small ovoid bacilli, which stained with the ordinary anilin dyes and were decolorized both by Gram's and Gabbett's methods.

Cultures of organisms were obtained which had all the characteristics of the bacillus described by one of us as the cause of an epidemic of hemorrhagic septicæmia among the carabao of the Government corrals during the past year. The chief characteristics of this organism were its polar staining, rounded ends, nonmotility, and occasional encapsulation. It grew invisibly on potato, did not produce gas in either solid or liquid glucose media, did not coagulate or acidify milk, did not form spores or liquefy gelatin, but did reduce nitrates and also gave the indol reaction. It was pathogenic for guinea pigs, causing death within twenty-four hours after intraperitoneal injection of 1 cubic centimeter. It was the *Bacillus bovisepiticus* of Kruse.

The tissues preserved for sectioning were embedded in celloidin, and sections from these were stained with hæmatoxylin and eosin, by Gram's method and by the carbol-fuchsin-acid one for tubercle bacilli. The stained sections showed a generally edematous condition of the parenchyma. The trabeculae were somewhat thickened and the fibrous tissue of the affected portion of the lungs was generally increased. The epithelial linings of the

bronchi were convoluted and hyperplastic, the cells being in many places three or four layers thick, and in the dilated lumina there were considerable accumulations of epithelial cells, polymorphonuclear leucocytes and a minimal amount of fibrin.

The air spaces of the affected lobes which were not involved in the consolidation were filled with a granular material with which there were occasional desquamated cells and leucocytes. The peri-bronchial tissue was the seat of well marked round cell infiltrations and the peri-bronchial connective tissue was considerably increased.

Sections of the small nodules of consolidation showed that the chief change in these was a coagulation necrosis. The centers of these areas were crowded with leucocytes and granular, cellular detritus enmeshed in fibrin. The walls of the air cells were perceptible as faintly pink-stained bands in which no nuclei or only shadows could be seen. As the periphery of these areas was approached, the lines became more distinct, and about the latter was a zone of congestion. There were no giant cells seen in any sections, and no "acidfast" bacilli could be found. There were, however, a number of very short bacilli between the cellular contents of the abscesses.

CASE II.

Calf No. 423 was received from Hongkong on January 6, 1904, and given a preliminary prophylactic dose of 100 cubic centimeters of anti-rinderpest serum. It was vaccinated on January 13, after which its temperature rose to 40° C. where it remained with slight remissions until January 19, reaching normal on the 20th. On January 21 it received 10 cubic centimeters of virulent rinderpest blood, and following this the temperature again rose and varied between 39.2° and 40.6° for the next eight days. On February 1 it received 50 cubic centimeters of virulent blood, and following this there was again a rise, which, however, was transient. From this time on the temperature remained within normal limits. Death occurred April 30, somewhat more than three months after the arrival of the animal at the laboratory.

During the last month or five weeks of its life a steady and gradual decline was evident. The animal lost weight in spite of a constantly fair appetite. Upon several occasions the blood was examined for trypanosomes, each time in vain. No cause could be found for the wasting. There was no cough, in fact no other symptoms than the gradual emaciation, and increasing weakness.

The day before death the animal was unable to stand but was lying in its stall in the hospital shed, eating the grass before it. It died the following night and was found in the morning in its sleeping position.

Upon opening the thorax a considerable amount of clear serofibrinous fluid gushed out. The peritoneal cavity contained no fluid and was apparently normal. The subpleural and mediastinal tissues were edematous and gelatinous in appearance. The prescapular glands and periglandular tissues were also edematous. The liver appeared pale but otherwise normal. There were a few petechiæ in the capsule of the spleen, which was of normal size and consistence.

The cecum contained a number of oesophagostomas and fasciolas.¹ There were no gastro-intestinal hemorrhages or ulcerations.

The lungs were the seat of a patchy hepatization, which involved about one-half of the entire pulmonary tissue, chiefly the apices and anterior lobes. In the hepatized areas, and perceptible to the eye and finger, upon the surface of the lungs, were small nodules. These were small and paler than the adjacent tissue, which had an almost purple color. Section of these organs showed that the fibrous tissue was generally increased in the affected portions, the trabeculae being quite prominent. The surfaces of cut sections showed mottled gray and red, the gray being most apparent in the centers of the lobules. Certain of these gray areas were quite dry and almost caseous, and were as large as a navy bean.

From these nodules cultures of a nonmotile polar-staining bacillus which did not stain by Gram's method and did not form spores were obtained on agar. The colonies in agar varied in size from 0.5 to 1 millimeter in diameter, were thick and opaque at their centers, were not chromogenic, had smooth, regular edges and were somewhat sticky and mucilaginous. On agar slants after twenty-four hours the growth was colonial, but with a tendency to confluence. The water of condensation was clouded.

On glycerin-agar the growth was more luxuriant, quite thin but almost opaque, and so moist that it was inclined to run down the surface of the slant.

Milk became just perceptibly acid after seventy-two hours, but without coagulation or reduction of the litmus. The growth on potato was invisible. In glucose broth and agar no gas was formed. In Dunham's peptone, to a liter of which 5 cubic centimeters of a 5-per cent solution of potassium nitrate had been added, a brilliant cholera red reaction could be obtained after twenty-four hours.² After seventy-two hours a pellicle appeared in the ordinary bouillon; the medium was diffusely clouded and a flocculo-gelatinous sediment was thrown down.

Of a twenty-four-hour-old broth culture, 1 cubic centimeter was injected into the peritoneum of a healthy guinea pig. The animal survived this treatment.

Cellodin sections cut from material fixed in Zenker's solution, and stained with hematoxylin and eosin, showed changes that correspond in all general respects with those described in the previous case.

CASE III.

Calf No. 415 was received at the laboratory on December 29, 1903, and given the usual prophylactic dose of 100 cubic centimeters of antirinderpest serum. The temperature remained normal until after vaccination, which

¹ Specimens of these parasites were sent to Chas. Wardell Stiles for identification.

² This method was suggested to us by Dr. W. B. Wherry. For some time we have all been troubled by the inconsistency of the peptone used in making Dunham's solution. Dr. Wherry, however, found that a constant reaction could be obtained by adding traces of nitrates to that fluid.

was done eight days later. Following this, on the third day, the temperature rose abruptly to 41.6° C. and then gradually fell to normal within the next week. On January 20, 1904, it was given a subcutaneous injection of 10 cubic centimeters of virulent rinderpest blood, following which there was a reaction of 1.4° C., after which the temperature fell to normal and continued so to the time of death on May 14, 1904, almost five months after the animal had been received. At no time were trypanosomes found in the blood. The clinical history in the case was not unlike that in the previous one; the salient points being gradual loss of weight in spite of retention of appetite, normal temperature, no cough, roughened and starchy coat, and loss of strength.

At autopsy the chief lesions, and in fact the only macroscopic ones, were in the lungs. There was not as much edema as in the previous case, and there was a less general pulmonary involvement. But here, as in the other ones, the apices and anterior margin of the lungs were partially solidified, mottled with red and gray, and filled with small, firm nodules, some miliary and some the size of a large bean. Some of these on section proved to contain a semifluid purulent material, while others were dry and gray.

Cultures were made as before from these nodular lesions and in this case two organisms were isolated, one *B. pyocyaneus*, the other a polar staining bacillus agreeing in general with the organism previously described, but varying from it in some cultural characteristics.

Morphologically, it was identical with the organisms from the previous cases. Culturally, variations were most marked in broth and on agar. On the latter, the growth was generally composed of colonies, but these were somewhat larger than the ones described for *B. bovisseptius*. The growth was much more luxuriant and whiter and there was a decided tendency to confluence on the part of the colonies.

In bouillon there was at first a diffuse cloudiness with no sediment and no pellicle. Later, a gelatinous sediment was deposited, a very delicate pellicle was developed, and the body of the medium became clear. There was also a deposition of floccules on the sides of the tubes.

The cholera-red reaction was obtained after twenty-four hours. Milk was unchanged, and there was an invisible growth on potato.

Intraperitoneal injection of 1 cubic centimeter of this organism killed a healthy guinea pig in less than twenty-four hours. At autopsy an acute hemorrhagic peritonitis was discovered, and the organism recovered.

CASE IV.

Calf No. 464 was received at the laboratory on January 12, 1904, and was injected with 100 cubic centimeters of antirinderpest serum. The following day its temperature was 40.1° , and on the succeeding it was normal and remained so for the next ten days. On January 20 it was vaccinated with vaccine virus, and following this its temperature rose, reaching a maximum, 41.1° , on the day after the vaccine was collected. Two days later it was again normal. On February 1, 1904, 10 cubic centimeters of virulent rinderpest blood was injected subcutaneously, and

following this the animal's temperature became quite irregular, reaching 40.5°, and with daily remissions of 1 to 2 degrees; but after twelve days the maximum had fallen to about 39.2° C. and with smaller remissions. This continued until death occurred, May 9, 1904, about four months after its arrival in Manila. Up to this time it was able to eat, but it gradually became emaciated and very weak.

At autopsy the lungs were found to be affected in a manner similar to the condition of the others described above. In this instance, however, the apices were alone affected over an area in each of about the size of the palm of a hand. These were purplish-red, edematous, and contained small areas of solidification, which appeared grayish or gray in sections. There was a fibrinous exudate over the pleural surface of some. The other organs showed no change other than mild parenchymatous degeneration. The body was anemic.¹

Cultures were made from the nodules in the lungs. On the original plate cultures two types of colonies were noted: One very small, similar to the typical colonies of *B. dovisepticus*, the other larger and with more tendency to spread, and upon slant cultures to coalesce. Both of these were studied.

The only cultural differences in these two strains were noted in agar, upon which one showed a greater inclination to spread and coalesce, and in broth in which one (464¹) caused a uniform clouding with a gelatinous sediment, while the other (464²) clouded the media less diffusely and formed flocculi, which were present not only in the body of the fluid but also on the sides and bottom of the tube. Both gave a brilliant indol reaction. Both were pathogenic for guinea pigs.

Sections for material fixed in Zenker's solution and imbedded in celloidin showed the same histologic picture as that described in above cases.

CASE V.

Calf No. 491 was received at the Serum Laboratory on February 24, 1904, and was given a prophylactic dose of 100 cubic centimeters of anti-rinderpest serum. A transient rise of temperature to 41.2° C. and a gradual return, during seven days, to normal followed this. On March 5, 1904, 5 cubic centimeters of virulent rinderpest blood was injected subcutaneously, upon which a scarcely perceptible rise of temperature lasting but twenty-four hours was noted. Four days later the animal was vaccinated and again the temperature rose, remained between 39° and 40° C. during the inoculation disease, and then gradually fell to normal. On March 25, 1904, a second injection, this time 50 cubic centimeters, of virulent blood was made, and this gave rise to a slight increase in temperature, which lasted but forty-eight hours.

During the following month the temperature remained above normal,

¹ In the small intestine there were some round worms, which were preserved and sent to Chas. Wardell Stiles for identification. In his report he says that they are in all probability a new species of *Hæmonchus*.

varying from 39° to 40° C., and only dropped immediately before death. The animal died on May 5, 1904, a little over two months after its arrival at the laboratory.

At autopsy, done about eighteen hours after death, the upper halves and the anterior lobes of the lungs were found to be of mottled purplish-red color, and almost completely consolidated. In the areas of consolidation were a few nodules, generally of a pyramidal shape with their bases in the pleura.

The pleural surfaces of these were covered with a thin fibrino-purulent exudate, which upon removal showed the yellowish color of the nodule. On section the nodules appeared to be abscesses, surrounded by consolidated pulmonary tissue, and containing a thick yellowish pus.

Throughout the consolidated portions of the lungs there were smaller, almost miliary, firm areas. In general these were in the centers of lobules about which the trabaculæ surrounding them were increased in size and quite prominent.

The other organs showed no marked changes.

Cultures from the abscesses upon glycerin-agar and in broth showed no growth.

Histological examination of sections showed approximately the same arrangement in and about the nodules as in the previous cases.

CASE VI.

Calf No. 453 was received at the Serum Laboratory on January 12, 1904, and given 100 cubic centimeters of antirinderpest serum. The next day its temperature was 40.2° C., after which it fell to normal, remained so for three days, and then gradually rose to 40.8°, afterwards again gradually falling, the course covering a period of one week. From this time until death the temperature remained normal, except for a transient rise after vaccination and one following injection of 50 cubic centimeters of virulent rinderpest blood. It died on April 27, 1904, about three and one-half months after coming to the laboratory.

At autopsy the pulmonary lesions were similar to those found in No. 491, though less extensive, being almost limited to the apices and the extreme exterior borders.

Plate cultures from the nodules after twenty-four hours at 37° showed three slightly variant types of colonies. Transplants were made from each type and studied as B. 453,¹ 453,² and 453.³

After carefully comparing their appearances in and on various media, no other difference could be seen than that of size. B. 453¹ was a trifle larger, generally, than the other two. All produced typical colonies—i. e., the ones which were larger, more opaque, and which showed a slight tendency to coalescence.

These various differences between these strains will be discussed later. Suffice it to say now, that, in general the organisms in their cultural and morphologic characteristics were identical with *B. bovissepticus*.

Histologic examination showed a condition that agreed with that seen in previous cases.

CASE VII.

Steer No. 383 was received at the Serum Laboratory on January 6, 1904, and was given 100 cubic centimeters of antirinderpest serum. Two days later it showed some indisposition, and upon examination the temperature was found to be normal, the pulse somewhat accelerated, respirations rapid and shallow, coat staring, appetite diminished, and rumination performed with indifference. Later the temperature became irregular with an evening rise which often reached 40° C. About ten days later, after the first examination, the animal developed a shallow, painful cough, which became more marked as the disease progressed. Tuberculosis, although up to that time unknown in these Islands, was suspected and the steer was killed sixty-one days after coming to the laboratory.

At autopsy a very widespread pathologic condition of the lungs was found affecting almost the entire extent of both organs. This consisted in a chronic bronchitis with bronchiectasis upon which a broncho-pneumonia had been engrafted. The bronchial walls were injected and covered with a sticky mucopurulent material. There were several bronchiectatic cavities, one the size of a goose egg, the others smaller, which had smooth fibroid walls within which were greenish-yellow muco-caseous masses of very foul-smelling material. The parenchyma of the lungs was studded with small areas of consolidation, some of which had undergone softening. The lungs were generally edematous. There were no marked changes elsewhere save congestion and cloudy swelling.

Cultures made from the small nodules at the time of autopsy showed no growths.

Tissues were preserved in Zenker's solution and embedded in colloidin. Sections from these materials were stained with hematoxylin and eosin, thionin, genetian violet (Gram and Weigert methods), and by the ones used to demonstrate tubercle bacilli.

The lungs on section showed an intense peri-bronchial infiltration, which was composed for the most part of cells of an epithelioid character with abundant protoplasm and vesicular nuclei. There was an increase in the connective tissue of these accumulations, which were also more or less regularly enclosed by fibrous capsules, making it appear as though the epithelioid accumulations had occurred between the mucous membranes and the surrounding muscular layers. Outside of the encapsulating layers were occasional lymphoid accumulations.

The mucous membrane of the bronchi was hypertrophied and corrugated—the cells being two or three layers thick and many of them vacuolated. The lumen was filled with mucous material in which numerous leucocytes were embedded.

In several places the mucous membrane on one side was hypertrophic, while on the other it was flattened and atrophied. Projecting into the lumen from the hypertrophied side were masses composed of small round cells and stroma, about whose internal edges were a row of large cells of peculiar appearance. One of these was an enormous cell whose protoplasm was very large in amount and whose nucleus was about 3 to 4

times the size of a poly-morphonuclear leucyte, and vesicular. The other ones were not as large, but were of the same type, except one large giant cell from whose position, and the arrangement, it is plain that it is formed by the coalescence of epithelioid (endothelial) cells. The remainder of the lumen was filled with cells similar to those described.

That the structures of the growth were granulomata might be seen in this same section, for the mixture of round epithelioid cells projecting into one large bronchus was well supplied with mature and immature blood vessels. There was no giant cell formation in these epithelioid or round-celled nodules. The only place, apparently, where this was found was on the lumen side of the projecting growths, and even here the occurrence was an exception.

The trabeculae of the lungs were increased in volume. The perivascular connective tissue was increased and the parenchyma was injected.

It certainly seemed that in some places there was a tendency to syncytial formation in the mucous membranes of the bronchi, especially in those in which the peripheral growth had encroached largely upon the lumens.

In other sections the profuse fibroid overgrowth was most well marked. In such bronchi, so nearly obliterated that nothing but remnants of mucous membranes were to be seen embedded, masses of round and vesicular cells occurred. Giant cells could be seen in these, but here, too, these were nearer the peripheral margin of the nodules, and they seemed in a certain degree to have some relation to the smaller bronchioles upon which the cellular accumulations had encroached.

In other sections nodules could be seen whose centers evidenced commencing rhexis.

The liver showed a remarkable degree of fibroid change, evidently originating about the bile ducts. The tissue, besides its formative elements, containing many cells with vesicular nuclei. New gall-duct formations were also noticed.

No organisms were seen in the sections that resembled glanders bacilli, and there were no "acidfast" organisms discovered.

CASES VIII AND IX.

Calves Nos. 486 and 405.—Neither in their clinical symptoms nor in their general condition did these animals show any marked symptoms of disease. Their temperature kept within normal limits except after virulent blood injections and after vaccinations. They had no cough. They ate up to the day of death.

In the case of No. 468, which became much weaker than did No. 405, appetite was present and the animal ate its fodder eight days after it had been unable to rise to its feet.

The lesions were comparable to those described in the other calves, but in both cases they were limited to the apices of the lungs. From the lesions of each animal an organism was obtained in cultures which was similar to the ones already described—viz, to *B. bovis septicus*.

The histologic examination showed approximately similar changes, although the processes were less advanced.

That these cases arose in the way Diekerhoff and Schneidemühl describe, there can be but little doubt. In certain animals, whose physical condition warranted slaughtering, we have noticed small areas of atelectasis, which might or might not have been congenital, a point which we have been unable to determine. In certain others we have found both acute and subacute bronchial changes, with no macroscopic parenchymatous changes. Whenever two such conditions coincided in the same animal the ideal opportunity would be afforded for the production of more serious pathologic complex, and we imagine that this is exactly what has happened in certain of these cattle.

It is an interesting feature of these cases that the bacilli of hemorrhagic septicæmia should be associated with so large a proportion.

Just what the duration of the disease has been in these animals can not be determined accurately. In the steer it was undoubtedly at least fifty days, and in the others it was probably thirty to ninety.

Infection has undoubtedly taken place in each case following the primary bronchitis, still later incipient pneumonic changes setting in.

Since the organisms present in the lesions proved to be the bacillus of hemorrhagic septicæmia, we may conclude that this is as common an inhabitant of the respiratory tract of animals here as it is in the United States.

Finally it may be said that from the above-mentioned facts that these cases are not examples of chronic infection with *B. bovissepticus*, but simply of an implanted infection in the course of other pathologic conditions, or perhaps in certain cases of a terminal infection. It seems probable, from our experience, that when primary infection with this organism occurs the disease runs a somewhat more acute course.

Another interesting fact was brought out in the bacteriologic investigation of these cases—namely, that the cultural characteristics of the *B. bovissepticus* are not constant. Even with organisms of approximately equal virulence the growths on and in media vary considerably (these are perhaps most marked in bouillon as the accompanying table will show), and with races of unequal virulence the differences are still greater.

No.	Pellicle.	Broth sediment.	Fluid.	Side of tube.	Gelatin liquefaction.	Agar.
464 ^a	Scanty	Flocculo-viscid	Diffusely cloudy	Precipitate	0	Large colonies; tendency to confluence.
464 ^b	do	do	Cloudy floccul	do	0	Fin dewdrop colonies; no confluence.
423	Whitish	do	Diffusely clouded	do	0	Moderately luxuriant, confluent growth.
415	Scanty	do	Clear	Precipitate	0	Confluent growth.
453 ^a	Whitish	Viscid	Diffusely clouded	do	0	Very delicate growth of fine colonies; no confluence.
453 ^b	do	do	do	do	0	Do.
453 ^c	do	do	do	do	0	Delicate growth of small colonies; no confluence.
487	Scanty	do	Clear	Precipitate	0	Small colonies; no confluence.
7	do	do	do	do	0	Very small colonies; no confluence.

No.	Milk.	Potato growth.	Glucose bouillon.	Peptone indol.	Gram's stain.	Motility.	Pathogenesis.	Remarks.
464 ^a	No change	Invisible	No gas	Positive	0	0	Guinea pig	Medium size; indol reaction brilliant.
464 ^b	do	do	do	do	0	0	do	Small indol reaction; brilliant.
423	Very faint acid	do	do	do	0	0	do	Largest of nine races.
415	No change	do	do	do	0	0	do	Medium size; indol reaction brilliant.
458 ^a	do	do	do	do	0	0	do	Small.
458 ^b	do	do	do	do	0	0	do	Very small.
458 ^c	do	do	do	do	0	0	do	Do.
487	do	do	do	do	0	0	do	Do.
7	do	do	do	do	0	0	do	Do.

It may be well to note here that, unless otherwise stated, the reaction of the media used is 1 per cent acid to phenolphthalein, and it was brought to this degree not by neutralizing with alkali and then adding hydrochloric acid, but by adding simply enough alkali to produce the right degree of acidity.

An excellent discussion of the cultural characteristics of the members of the bacilli of hemorrhagic septicemia will be found in Moore's book on "The Pathology of Infectious Diseases of Animals."

A fuller discussion of the variations of *B. bovisepiticus*, especially as regards pathogenicity, must be left for further study. From the few facts at the present time available we are not willing to draw conclusions.

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PART III.

REPORT ON PINTO (PAÑO BLANCO).

By PAUL G. WOOLLEY, M. D., *Director of the Serum Laboratory.*

Under the terms paño blanco, pinta, pinto, caraté, mal pintado, mal de los pintos, mal del pinto, peint, cute, cativi, quirica, pannus, carateus, and the spotted disease of Central America, is included a group dermatomycoses, characterized by peculiar nonpigmented patches on the skin, in the scales from which hyphæ or spores or both, of a mold-like fungus are found, which resemble in some cases *Penicilium*, in others *Aspergillus*, in still others *Monilia*.

Heretofore this epiphytic disorder has been reported from Mexico and Central and South America; another disease resembling it in some respects has been observed by Legrain in North Africa, and by Sandwith in Egypt, but, so far as I know, no previous report has come from the Philippine Islands.

The case which I wish to record is not the only one which I have seen in Manila, but it is the only one from which I have been able to obtain specimens for examination. All of the affected persons whom I have noticed have shown only the white variety, of which the following case is an example.

The history of the case is as follows: The patient was a Filipino laundryman, 15 years old, and in good health. There was no similar disease in any of his immediate family.

Upon inspection it was noticed that there were pinkish white patches, irregular in size and shape, on the ankles, dorsa of the feet, shins, knees, elbows, hands, wrists, and one on the right shoulder. This last-mentioned lesion the boy says was the one first noticed. The largest ones were over the external malleoli of the

ankles. These, the boy says, appeared after the one on the shoulder. The patches on the knees and elbows occurred later. None of these patches were of the same shape or size, nor were they definitely defined, but they shaded from their clear white centers to the normal brown of the skin. Neither were the lines of extension regular, so that the outlines of the patches were irregular and crenated. About the larger areas were smaller ones, some barely visible and of a faint pinkish white or very light-brown color.

On palpation it was evident that the skin over the larger patches was slightly rougher than the normal and that it felt somewhat thicker. The palpating finger could detect no abnormal variation in the covering of the smaller spots. There was but a minimum amount of scaling, and there was some itching.

The rate of extension had been extremely slow, for in three years the largest patch had a diameter of but 7 by 5 centimeters.

When asked regarding the cause, the boy said that the first spot came from carrying laundry baskets on his shoulder, and that the other ones followed traumata of one kind or another. There were no lesions on the palms of the hands or soles of the feet.

From one of the larger lesions on the ankle scrapings were made and examined in a solution of caustic potash (25 per cent). Among the epithelial cells branching, segmented hyphæ were seen forming a coarse mesh work. The mycelium was somewhat finer than that of *Trichophyton*; it was in general evenly refrangent, but in places beaded or granular. The spores were darker in color than the rest of the organism and less refractile. An occasional fructification was found in the smears, and in these the arrangement of the spores was like that of *Penicilium*. When treated with dilute fuchsin the spores were stained a very deep red. The hyphæ showed an inner segmented arrangement with a continuous inclosing capsule.

There was nothing in any of the preparations to suggest the description of *Gastambide*. The mycelial filaments were usually long, branched, and terminated in a bunch of spores. The description given by Montoya y Flores seems to apply more accurately to the fungus of this case.

There can be no doubt of the nature of the disease. The clear white spots with almost normal looking skin can be confused with no other skin affection with which I am acquainted. Diseases caused by trichophytons are extremely common in Manila and are

known generally as "dhobie itch," which is so common in the natives that in thirty cases of skin disease taken at random in Bilibid Prison twenty-four showed trichophyton filaments in caustic potash preparations.

It is possible that a brown pinta might be confused with pityriasis versicolor should the small patches occur on the face where it is said that the latter may occur. However, in the present case the clear white color of the irregular patches, the presence of sensation and of itching, together with microscopic findings, are enough to assure a correct diagnosis.

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PART IV.

NOTES ON ANALYSIS OF THE WATER FROM THE MANILA WATER SUPPLY.

By CHARLES L. BLISS, B. S., *Chemist.*

In December, 1903, it was suggested by the Superintendent of Government Laboratories and the Director of the Biological Laboratory that a systematic investigation, both from a chemical and bacteriological standpoint, be made of the water supplied the city of Manila. After careful consideration it was decided that this investigation should consist of the usual sanitary chemical analysis and a count of bacteria, these to be made at regular intervals of one week and to extend over a period of several months. Later it was decided to examine the water for the presence of amebas and also to extend the investigation so as to include samples taken from the course of the watershed.

There were several reasons why such examinations were desirable:

(1) Up to that time no chemical analysis of the city water had been made at the laboratory, and frequent requests for statements in regard to the water had to be denied.

(2) It was supposed the water would vary considerably from time to time—not only from one season to another, but even from day to day. It seemed very probable that during the rainy season especially it would be subject to great changes within the course of a few hours, and that the results would be markedly different from the ones obtained during the dry, hot months preceding. This seemed all the more probable if any conclusions can be reached from the appearance of the water on different days. While ordi-

narly it is quite clear, a few hours after a rain it shows turbidity, which is very marked after a heavy storm. The watershed contains a quantity of suspended matter in the form of a fine silt, most of which gradually deposits when it is allowed to stand for a short time. This would naturally lead to the supposition that a variation could also be expected in the soluble constituents and in the number of bacteria.

(3) Although numerous bacteriological examinations have been made of the city water since the organization of the Biological Laboratory, these had not previously been made at regular intervals, but only at random as opportunity offered; moreover, they were undertaken for different purposes. At times merely a count of bacteria was needed; at others, a search for certain specific organisms was undertaken, so that no continuous record was made of the number of organisms found in the water.

(4) Amebas had been frequently found in the water in past times,¹ but it was not definitely known whether or not they were always present. With the prevalence of amebic dysentery in the city it was especially desirable to know whether these organisms were constantly present in the water.

The investigation was begun on December 14, 1903, and continued during seven months. It extended over the greater portion of the cooler weather, the hot season, and the beginning of the rainy season. At first weekly examinations were made; but after three months, there having been but little variation, examinations were made every alternate week. All samples were taken from a tap in the laboratory except on the following days: February 23, from El Deposito; February 29, from the Mariquina River at Santolan, and March 7, four samples from the Mariquina River (the watershed) at different points.

The taking of the samples (with the exception of those from El Deposito and Mariquina River), the methods of manipulation, the apparatus and chemicals were as nearly uniform as possible throughout the entire period, Monday of each week being selected as the day for doing this work. The last sample was analyzed on

¹Dr. Strong called attention to the presence of amebas in the water and to its unfitness for drinking purposes in his annual report as Director of the Biological Laboratory in 1902 and 1903. See Report of the Superintendent of Government Laboratories for 1902 and 1903.

July 15, for the reason that there had been an excessively heavy rainfall on the four days preceding, during twenty-seven hours of which time $17\frac{1}{2}$ inches of rain had fallen, the city and outlying districts having been completely under water from the evening of the 12th till a day or so later. A few weeks earlier an examination made eight or nine days after a rather severe typhoon showed results very nearly the same as those which had been obtained throughout the series; it was therefore thought best to take a sample very soon after the flood, rather than wait until the following week. It might be remarked that even in this instance there was but very little variation from the usual results.

Two samples were taken from one of the laboratory taps each Monday morning, after the water had been allowed to run for at least one-half hour; one was retained for chemical analysis, the other, with the usual precautions to prevent contamination, was sent to the Biological Laboratory.¹ Both examinations were begun immediately. The sample for chemical analysis was collected in a 3-liter, glass-stoppered bottle, which was first rinsed thoroughly, filled and emptied two or three times, and then filled to the neck; the stopper was well rinsed and immediately inserted. This bottle was used for no other purpose throughout the series. The determinations of nitrites, nitrates, ammonia, and oxygen consumed were started at once so as to obtain results representing the true condition of the water before any changes due to oxidation, reduction, or bacterial action could vitiate it, all necessary precautions to prevent contamination by laboratory fumes being taken. As the results in chlorine and hardness would not be affected by any changes which might take place in the water, and as the residues would be but very little if at all altered, these determinations were deferred until opportunity to make them was at hand; but in every instance they were undertaken before the end of the week. In order to represent the water as it actually came from the pipes, all analyses were made with the unfiltered liquid.

The chemical analysis consisted of the determination of total residue; fixed residue; loss on ignition; nitrogen in the forms of

¹ The counting of the bacteria was done by Mr. Clegg, of the Biological Laboratory, and the determination of the presence of amebas was also done by Mr. Clegg in conjunction with Dr. Musgrave, who has compiled his results in Bulletin No. 18, Bureau of Government Laboratories, Biological Laboratory.

nitrites, nitrates, free and albuminoid ammonia; oxygen consumed; chlorine; also hardness. As some of the methods of manipulation were different from those usually employed, a brief description is given.

Total residue was determined in a platinum dish easily holding 100 cubic centimeters, by evaporation on the water bath, and heating for thirty minutes at 95° after drying. The dish, after the weighing of the total residue, was heated uniformly to low redness for three or four minutes. It was then placed in the desiccator and weighed as soon as cold. There was practically no change in the appearance in any sample on heating; at most only a very slight darkening and but little odor were perceptible, indicating the presence of only small amounts of organic matter. The loss on ignition was therefore due largely to decomposition of carbonate, as the heat was not sufficient to volatilize any chlorides.

Nitrites.—The reagents were prepared as follows:

(a) Eight grams of naphthylamine hydrochloride were dissolved in water, 8 cubic centimeters of concentrated hydrochloric acid added, and the solution diluted to one liter.

(b) Sulphanilic acid, a saturated solution in water containing 5 per cent concentrated hydrochloric acid. The test was made by placing 50 cubic centimeters of the water in a Nessler tube, adding 1 cubic centimeter of each of the above solutions, and mixing well by gently shaking. After thirty minutes the color was noted. In no instance did the depth of color indicate more than a very faint trace of nitrites, and very frequently none developed. Therefore a quantitative estimation could not be made.

Nitrates.—The aluminum reduction method was employed. After a few hours, when the reaction was completed, the ammonia formed, together with the free ammonia originally present in the water was estimated directly by the Nessler process; the free ammonia (determined in another portion of the sample) was deducted, the remainder being the ammonia formed from the nitrates. In the present series the determination of nitrates was begun within a few minutes after the sample was received, and the Nesslerizing was done on the following day. If nitrites are present in appreciable amount an allowance should be made for them.

A little more than 50 cubic centimeters of the sample were placed

in a 250 cubic centimeter glass-stoppered bottle; 2 cubic centimeters of sodium hydroxide of 33 per cent strength and 2 grams of aluminum filings were added, and the loosely stoppered bottle allowed to stand at room temperature until the next day. The solution was then filtered, with all precaution, into a tube, filling it to the 50 cubic centimeter mark. This solution was then Nesslerized, the necessary correction for free ammonia being made.

Free ammonia.—A round-bottom flask of 1 liter capacity with short neck was connected with a condenser 1 meter in length, the Nessler tube being slipped over the other end. On the day the sample was received the distilling flask was rinsed with distilled water; about 200 cubic centimeters of a solution of distilled water containing 1 gram of sodium carbonate were then added, and the greater part of the water distilled off, until the apparatus was free from ammonia. After cooling, 500 cubic centimeters of the sample were added to the residue, and the distillation continued at such a rate that a Nessler tube was filled to the 50 cubic centimeter mark in about ten minutes. Three tubes of 50 cubic centimeters each were collected and Nesslerized. As a matter of fact one tube would usually have been sufficient, at most two; for the third one never showed more than a slight trace of ammonia, and often none at all.

Albuminoid ammonia.—Fifty cubic centimeters of alkaline permanganate (8 grams of permanganate, 200 grams of potassium hydroxide, and 1,100 cubic centimeters of water, evaporated to 1 liter) were now added to the contents of the flask and distillation was continued, four tubes of 50 cubic centimeters each being collected. The distilling apparatus was used for no other purpose throughout the series, and it was always well protected from fumes.

Nesslerizing.—The Nessler solution was prepared according to the usual method and the standard solution of ammonium chloride contained (0.03812) gram of pure ammonium chloride in one liter. One cubic centimeter represents 0.00001 gram of nitrogen.

The distilled water in the laboratory was found to be free from even the slightest perceptible trace of ammonia; it was tested each time. A number of Nessler tubes were thoroughly rinsed with this ammonia-free water, then filled to the 50 cubic centimeter mark; portions of the standard ammonium chloride solution were measured in from a normal capillary pipette reading accurately

in hundredths of a cubic centimeter. The amounts used were 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.5, 2, and 2.5 cubic centimeters. Two cubic centimeters of the Nessler reagent were added to each, and also to the tubes containing the ammonia (free, albuminoid, and that from the nitrates). After allowing thirty minutes for development of the color, the comparisons were made. The results were expressed in terms of nitrogen per million.

Oxygen consumed.—The solution of pure potassium permanganate contained exactly 0.3953 grams in 1 liter. One cubic centimeter represented 0.0001 gram available oxygen, and the solution of pure ammonium oxalate was of exactly equivalent strength. These two solutions were kept in a dark closet and were standardized from time to time.

One hundred cubic centimeters of the water were measured into a 200 cubic centimeter Erlenmeyer flask from a pipette; 2 cubic centimeters of pure concentrated sulphuric acid were added, and then 10 cubic centimeters of the permanganate solution; the flask was then suspended in a boiling-water bath for thirty minutes. It was then taken out and 10 cubic centimeters of the oxalate solution were immediately added. The solution became colorless within a minute or so, and was then titrated with the permanganate till a faint pink color appeared, which remained permanent for a few moments. This determination was made in duplicate, two flasks being carried through at the same time. In no instance did the two titrations vary more than 0.05 cubic centimeters, and usually they were identical. For convenience, the titrations were made with a very narrow pipette graduated in 0.05 cubic centimeters. Results were expressed as parts of oxygen consumed per million.

Chlorine.—A solution of pure, recrystallized and dried silver nitrate was made containing 4.7940 grams in 1 liter. One cubic centimeter represented 0.001 gram of chlorine. A 5-per cent solution of potassium chromate freed from chlorides by precipitating with silver nitrate was used. The chlorine determinations were made in duplicate. Two portions of 250 cubic centimeters each were measured into casseroles of about 300 cubic centimeters capacity, carefully evaporated to about 50 cubic centimeters, 1 cubic centimeter of the chromate solution added, and the mixture titrated with the silver solution. By using one as a comparison

the first change in color from a pure to a reddish yellow was noted very sharply, and the two results never varied by more than 0.05 cubic centimeter; usually they were identical. A pipette similar to the one employed in the titration with permanganate was used. Results were expressed in terms of chlorine per million.

Hardness.—A solution of pure calcium chloride was diluted so that 1 cubic centimeter contained 0.0002218 grams, or represented the equivalent of 0.0002 gram of calcium carbonate. Fifty cubic centimeters of this solution should require exactly 14.25 cubic centimeters of standard diluted soap solution in order to form a lather which covered the surface of the liquid and persisted for five minutes. The soap solution was standardized each time and the proper value obtained, 50 cubic centimeters of the above calcium chloride being used for this purpose. This was compared with 50 cubic centimeters of the sample treated in the same manner, and the degree of hardness determined by reference to published tables. In the two or three instances, when the results varied from those usually obtained, and in all cases where any doubt as to the exact end-point existed the determinations were repeated.

Analyses of the Manila water supply.

[Results are given in parts per million.]

Laboratory No.	Location.	Date.	Total residue.	Fixed residue.	Loss on ignition.	Nitrites.	Nitrates.	Free ammonia.
1739		Dec. 14, 1903	220	190	30		0.150	0.0079
1745		Dec. 21, 1903	181	148	33		.271	.0049
1756		Dec. 28, 1903	188	152	36		.100	.000
1780		Jan. 4, 1904	188	142	46	0	.112	.008
1781		Jan. 11, 1904	191	160	31	0	.080	.002
1803		Jan. 18, 1904	179	148	31	0	.110	.006
1821		Jan. 25, 1904	176	152	24	0	.198	.002
1840		Feb. 1, 1904	178	152	26	0	Trace.	Trace.
1880		Feb. 8, 1904	162	142	20	(1)	.100	Trace.
1922		Feb. 15, 1904	168	152	16	0	.138	.002
1944	Deposito	Feb. 23, 1904	168	145	23	0	.274	.006
1996	Santolan	Feb. 29, 1904	164	138	26	0	.292	.028
2025	Mariquina River	Mar. 7, 1904	160	134	26	0	.124	.036
2026	do	do	153	127	26	0	.139	.021
2027	do	do	174	147	27	Trace.	.112	.028
2028	do	do	173	145	28	0	.136	.024
2069		Mar. 14, 1904	167	138	29	(2)	.220	Trace.
2157		Mar. 28, 1904	173	147	26	(2)	.220	Trace.
2225		Apr. 11, 1904	174	138	36	Trace.	.158	.002
2303		Apr. 25, 1904	165	136	29	Trace.	.200	Trace.
2352		May 9, 1904	180	145	35	(1)	.158	.004
2385		May 23, 1904	169	135	34	(1)	.120	Trace.
2434		June 6, 1904	196	150	46	(2)	.360	Trace.
2464		June 20, 1904	174	138	36	0		Trace.
2487		July 5, 1904	178	150	28	0	.360	Trace.
2511		July 15, 1904	191	159	32	0	.220	Trace.

Analyses of the Manila water supply—Continued.

[Results are given in parts per million.]

Laboratory No.	Location.	Albumin ammonia.	Oxygen.	Chlorine.	Hardness.	Bacteria.	Amebas.	Remarks.
1730	-----	0.078	1.80	2.13	85.7	400	--	Heavy rain during few days preceding; water very turbid.
1745	-----	.073	1.85	2.23	85.7	550	—	Do.
1756	-----	.081	.875	2.60	99.0	600	—	Water distinctly turbid.
1780	-----	.052	.86	2.40	94.3	460	—	Water slightly turbid.
1781	-----	.064	.85	3.00	101.5	350	—	Do.
1808	-----	.038	.65	3.04	94.3	200	—	Do.
1821	-----	.044	.65	3.00	87.1	200	—	Do.
1840	-----	.048	.90	3.00	95.0	150	—	Water almost perfectly clear.
1880	-----	.046	.90	2.60	104.0	125	+	Do.
1922	-----	.044	.85	3.20	95.0	150	+	Do.
1944	Deposito.	.052	.95	3.30	109.0	120	(⁴)	
1996	Santolan	.086	1.25	3.40	97.0	112	0	
2025	Mariquina River.	.100	1.60	3.60	97.0	-----	+	All contained deposit, apparently organic matter and silt. Determinations of residue were made with filtered water. Each gave slight dark color and odor on ignition.
2026	do	.080	1.50	3.20	93.2	208	+	Do.
2027	do	.082	1.07	3.60	93.2	105	+	Do.
2028	do	.080	1.85	3.60	94.8	267	+	Do.
2069	do	.048	1.00	3.88	95.0	120	+	
2157	-----	.052	.82	4.14	101.8	120	0	
2225	-----	.062	1.07	3.80	97.8	108	+	
2308	-----	.048	1.20	4.40	95.0	125	+	
2352	-----	.064	1.30	4.20	98.0	175	+	
2385	-----	.042	1.05	4.40	92.0	180	+	
2434	-----	.074	1.60	4.20	91.0	206	+	Heavy rains just previous; water turbid; slight sediment. Slightly dark color and odor on ignition.
2464	-----	.040	-----	35.0	83.0	135	+	No rain immediately preceding.
2487	-----	.050	1.70	2.80	71.4	450	+	Very turbid.
2511	-----	.068	2.20	3.16	56.8	(⁵)	+	Heavy rains. Very turbid. (Flood.)

¹ Very faint trace.² Faint trace.³ Distinct trace.⁴ Not sufficient sample. A sample taken following week, bacteria, 100; amebas, 0.⁵ Rapid growth over surface prevented count.

The counts of bacteria and determinations of the presence of amebas were made by Dr. W. E. Musgrave and Mr. M. T. Clegg, both of the Biological Laboratory.

On examining the above table the following maxima and minima appear during the period covered by the report :

	Minimum.	Maximum.
Total residue.....	158	220
Fixed residue.....	127	190
Loss on ignition.....	16	46
Nitrites (N).....	0	Trace.
Nitrates (N).....	Trace.	.86
Free ammonia (N).....	0	.088
Albuminoid ammonia (N).....	.081	.100
Oxygen consumed (O).....	.65	2.20
Chlorine (Cl).....	2.18	4.40
Hardness.....	58.8	109

As will be seen despite the great variation in the weather conditions the differences in analytical results were not very great. At times the chlorine ran as high as 4.40 indicating some contamination, but these maxima were only transitory. However, a water may show a very high degree of purity, so far as this can be determined by chemical analysis, and yet be unfit for drinking purposes because of either the large number of micro-organisms which it contains or because of their nature; so that it may be possible to convey the etiological factor of typhoid fever, cholera, dysentery, etc., by a drinking water which chemically would be pronounced unobjectionable. The chemical analysis may indicate a probable pollution with sewage or with other matter which may be suspicious or dangerous at all times, and it may condemn such a water. However, in the case of the Manila supply the long series of analyses gave such results that no one would be justified, even at the worst, in stating from a chemical standpoint that this water was either injurious or deleterious to the public health. For this reason, as has been repeatedly pointed out by others, a bacteriological examination is essential.

However, in glancing over the number of bacteria it will be seen that the maximum, on December 28, was 600, and this large number was quite unusual, the average being below 250 organisms to 1 cubic centimeter and in many cases even below 150. Bacteriologically, therefore, the water may not be regarded as very suspicious, especially since the general series of determinations did not demonstrate pathogenic organisms to be present, and indeed typhoid fever is of but rare occurrence in Manila. During the cholera epidemic it does not seem likely that any of the cases could have been referred to direct infection from the Manila water supply.

A factor however, which, apart from the chemical and bacteriological analysis, is the most important, is shown in the last column, where it is demonstrated that amebas, whether pathogenic or not, are almost constantly present in the water supply, and Dr. Musgrave and Mr. Clegg, of the Biological Laboratory, in Bulletin No. 18, "Amebas: Their Cultivation and Etiologic Significance," have shown that amebic dysentery can be sometimes produced in monkeys by cultures made from the water supply. Neither a chemical analysis nor a bacteria count will demonstrate the presence of these dangerous factors of disease, and consequently, in the Tropics at least, if we wish to obtain a fair idea of the condition of the water, a determination of the presence or absence of amebas is necessary.

The ordinary sanitary analysis does not include an examination for substances which in themselves are injurious. The quantities of ammonia, nitrate, chlorine, etc., found in an ordinary water are harmless—they are simply indicative of a possible pollution, but before judgment can be passed in regard to the sanitary analysis, the source of the water, the geological conformation of its surroundings, and so forth, must be taken into consideration. An amount of chlorine, for example, which would be perfectly normal in water from one locality might indicate contamination with sewage in that from another. The total residue in this series was always below 200 parts per million excepting on one occasion which was after a heavy rain.

Very little darkening of the residue on heating was noted in any sample; sometimes there was none, so that in this respect no criticism can be made of the water. The presence of nitrites in measurable quantity is sufficient ground for condemning a water as a rule, for nitrites indicate bacterial action. However, in this water nitrites were frequently absent altogether, and only on one occasion were there more than a trace, this occurring immediately after a heavy rain.

The amount of nitrates was always very low. The highest amount of free ammonia found was 0.008 per million, excepting in five samples taken from the Mariquina River itself where the results varied from 0.0021 to (0.036), so that by the time the water from the river reaches Manila the ammonia has largely become oxidized to nitrate. This is borne out by the fact that the amount of nitrate in those samples which were taken on March 7 was

lower than the usual quantity obtained. Albuminoid ammonia, as a rule, was also quite low. None of these factors, therefore, would indicate a contamination of the river water.

The same may be said of the amount of oxygen consumed. No figure exceeding 2 was found excepting in one instance, and that was three days after the great flood in July. The amount of oxygen consumed seemed to be greater immediately after heavy rains.

The low results obtained in the investigation of chlorine are favorable indications as to the quality of the water. One would expect rather higher chlorine values in the waters of the Philippine Islands owing to the proximity to the sea, but this is not as a rule the case, as analyses of waters from other localities have demonstrated.

After heavy rains more or less insoluble suspended matter, both inorganic and organic, is rinsed into the supply. However the turbidity of the Manila water is generally due to a very fine silt which has its rise in some of the clay beds at the source of the river, and is for this reason harmless. Therefore the analyses made in the Chemical Laboratory show that the water supply of the city of Manila is of a very good quality, but the constant presence of amebas, as demonstrated in the Biological Laboratory, render the water unsafe for drinking purposes unless it is boiled.

23394—4

PART V.

FRAMBOESIA: ITS OCCURRENCE IN NATIVES OF THE PHILIPPINE ISLANDS.

By PAUL G. WOOLLEY, M. D., *Director of the Serum Laboratory.*

In a recent visit to Benguet Province, in the central part of northern Luzon, I became interested in a peculiar disease which was called "Lepra" by the native Igorrotes, this term having been taught them by the Spaniards. Inasmuch as I was entirely unprepared for a complete study, and because it is extremely difficult to obtain any history or to make complete examinations of these people, the following report will be meager, but it is interesting, since I feel certain that the disease is one closely related to if not identical with framboesia.

In Baguio, Benguet, I saw, with Dr. Thomas of the Civil Sanitarium, two cases; one, a woman aged about 35 years, and her son, the latter of some 15 years, both of whom presented small raspberry growths upon the face. In the mother, these were situated at the corners of the mouth; in the son, in the nasolabial folds, and they much resembled the growth pictured in the New Sydenham Society's Atlas (Fasc. XIV, Pl. B. fig. 8). Both of these persons showed pigmented scars on the neck and face. At a later time I searched for these two people in their native town of Agno, Benguet, but was unable to find them.

The case from which I procured the tissue which I will describe below, I saw one morning as Mr. Barron—the sanitary inspector of the province—and I were returning from a long trip in the mountains. As we stopped to rest at a little native village, we

noticed that several of the children had peculiar looking, sluggish ulcers on their legs, necks, or bodies, and also showed signs of considerable anemia. We asked concerning this affection and were told that the Spanish called it "Lepra," and that many people had it. This was all we could learn, except that the sores were not painful, though they showed the effects of scratching, and that they eventually healed. One grown person, a woman, was found who had similar lesions on her neck. Whether she had others elsewhere we were unable to discover. One lesion on the neck (see Sydenham Society's Atlas, Fasc. XIV, Pl. LXXXV, upper left-hand corner of lower figure) was a shallow ulcer with a firm grey base, a well-defined, firm margin, a slight yellowish secretion, not surrounded by any appreciable induration, not painful, and situated on the neck just below the angle of the jaw. This was excised and preserved in commercial alcohol. Near it were some pigmented areas, somewhat darker than the normal skin, not appreciably thickened, and possessed of sensation, and which the woman said were at the sites of healed ulcers similar to the ones we saw. (New Syden. Soc. Atlas, Pl. LXXXV.) An infant which the woman carried had similar lesions on its legs, face, and neck. Another child of the same woman, which, however, we did not see, was said to be afflicted in the same manner.

The gross appearance of the lesions would lead one to think of leprosy, tuberculosis, epithelioma, syphilis, or yaws. Leprosy I think may be excluded. There were no cases of outspoken leprosy among the persons of the pueblo in which these persons lived. There were no anesthasias or leucoplakias in any of the cases examined. The histologic examination was negative. Tuberculosis could be excluded since neither the lesions nor the scars had the classic appearance of lupus, nor was the histologic evidence sufficient to support such a diagnosis. Epithelioma could only be excluded by microscopic examination. All in all the cases seemed most like syphilis. None of the people of the pueblo showed outspoken signs of this disease, although, as stated, a complete examination could not be obtained. The inhabitants of this region generally are rigidly moral and rigorous punishment is inflicted upon any who overstep the bounds. But while they live morally clean lives, their physical surroundings are filthy, which may account for the modification of the framboesial lesion and the predominance of

infection. Treatment, of course, has not been tried, so one can not say what effect mercury or iodides might have, but from the evidence that I have at present I incline to the idea that the cases seen were examples, not of syphilis, but of frambœsia.

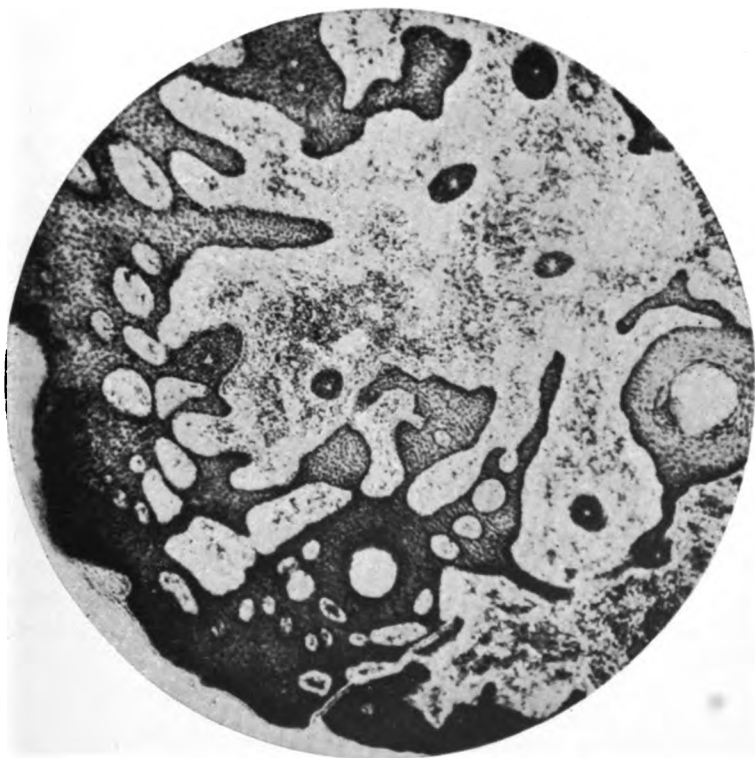
The tissue was embedded in celloidin, and the sections which were made later were stained with hæmatoxylin and eosin, Unna's polychrome blue, Gram's stain, and by the tubercle bacillus methods. Microscopic examination showed that the lesion consisted of a marked hyperplastic acanthosis, with a round-celled infiltration of the underlying, and especially of the perivascular, connective tissues. The first impression was that one was dealing with an epithelioma, but closer examination showed this to be a delusion. The acanthus layer of the skin was thickened and prolonged in strands and columns of bizarre shapes. In many places in this hyperplastic epithelium there were larger or smaller islands of connective tissue, each, apparently, representing the path of a blood vessel. In the centers of such areas and about the vessel were collections of small round and plasma cells, but this small-celled infiltration was most marked in the larger strands of the sub-malpighian connective tissue. Here the increase of these formative elements was remarkably prominent, and there was throughout the sections the same perivascular arrangement. Within these areas there were occasional leucocytes; there were also fibroblasts in varying stages of development, and a number of plasma cells were present within the round cell accumulations. At the site of the ulceration the structure of the lesion was modified by the destructive process. Here all the layers were invaded by a multitude of polymorphonuclear leucocytes, the blood vessels were widely dilated, and there was a certain amount of superficial degeneration, but the structure of the lesion in the not degenerated parts was still perceptible. So far as the arrangement of the layers of the skin was concerned there was no distortion. In many parts of the sections a peculiar appearance was seen which gave one the same impression as that produced by the scales of a fish, or by the overlapping of shingles upon the roof of a house. This was apparently due to the fact that certain of the acanthus cells took a more intense stain on one side. In this phenomenon (seen best in polychrome blue specimens), the nucleus did not participate. In none of the sections, and these included the whole of the lesion studied, were there

any giant cells, tubercle of lepra bacilli, and no evidence of cell inclusions was seen.

As for the occurrence of such lesions in frambœsia, and their distribution, little can be said excepting as quotations from authors who have had considerable experience with the disease. Manson says, in discussing the distribution of the yaws, that they may be scattered over the entire body, or the crop may be limited to one or two growths, or they may be confined to a circumscribed region of the skin. Moreover, there may be successive crops evolved, especially when the person affected is debilitated. Morris remarks that the disease in adults is more chronic than in children. When the yaw develops normally it does not ulcerate, but Manson says that the tumors instead of being absorbed, may break down and ulcerate, the ulceration usually being confined to the yaw itself, although it may go deeper and give rise to extensive sores. With the development of the deeper and more extensive forms of ulceration, the typical lesion of frambœsia may disappear for a time, or perhaps permanently. If such is the case, the ulcerations are said not to be infective and to not communicate the disease, although they may persist for years. Nicholls, quoted by Mason, states that ulceration occurs in about 8 per cent of the cases. There is no histologic description of the variety of the lesion which I have encountered to which I can refer, though from Unna's and Charlouis's description of the typical yaw it is but a modification of the latter. These writers speak of the true yaw as a cutaneous plasmoma complicated by epithelial hyperplasia and hyperkeratosis. Except that the lesion described above is not raised it certainly corresponds in many details with Unna's and Charlouis's description.

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 MORRIS. Diseases of the Skin. Philadelphia, 1898.
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SECTION STAINED WITH HÆMATOXYLIN AND EOSIN. (ZEISS COMP. OC. 6, OBJ. A. A.)

This shows the hyperplasia of the acanthus layer of the skin, the dilated blood vessels, and the perivascular accumulations of small round and plasma cells. Photomicrograph by Martin.

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(Continued from second page of cover.)

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